Morning-evening variation in human brain metabolism and memory circuits

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Submitted 26 July 2012; accepted in final form 25 November 2012

Shannon BJ, Dosenbach RA, Su Y, Vlassenko AG, Larson-Prior LJ, Nolan TS, Snyder AZ, Raichle ME. Morning-evening variation in human brain metabolism and memory circuits. J Neurophysiol 109: 1444–1456, 2013. First published November 28, 2012; doi:10.1152/jn.00651.2012.—It has been posited that a critical function of sleep is synaptic renormalization following a net increase in synaptic strength during wake. We hypothesized that wake would alter the resting-state functional organization of the brain and increase its metabolic cost. To test these hypotheses, two experiments were performed. In one, we obtained morning and evening resting-state functional MRI scans to assess changes in functional brain organization. In the second experiment, we obtained quantitative positron emission tomography measures of glucose and oxygen consumption to assess the cost of wake. We found selective changes in brain organization. Most prominently, bilateral medial temporal regions were locally connected in the morning but in the evening exhibited strong correlations with frontal and parietal brain regions involved in memory retrieval. We speculate that these changes may reflect aspects of memory consolidation recurring on a daily basis. Surprisingly, these changes in brain organization occurred without increases in brain metabolism.

functional magnetic resonance imaging; functional connectivity; resting state; sleep; memory; consolidation; diurnal; circadian default mode network; positron emission tomography; metabolism

SEVERAL HYPOTHESES ATTEMPT to explain why we must sleep. One prominent idea is the synaptic homeostasis hypothesis, which suggests that a major function of sleep is the regulation of synaptic weights: over the course of a day, learning and experience cause an increase in the brain’s synaptic density, which is pruned each night during sleep (Gilestro et al. 2009; Tononi and Cirelli 2003). An important corollary of this hypothesis is that an increase in synaptic density should boost the metabolic cost of brain function, because the majority of that cost originates in the synapses. Over the course of months and years, both declarative and nondeclarative memories are consolidated from medial-temporal regions to neocortical areas (Squire and Alvarez 1995), under anesthesia (Martuzzi et al. 2010), or through independent component analysis (Zhang and Raichle 2010).

The experience of forming new episodic or procedural memories often induces electroencephalography (EEG) changes during sleep on the following night (De Koninck et al. 1989; Gais et al. 2002; Huber et al. 2004; Massimini et al. 2009). Over the course of months and years, both declarative and nondeclarative memories are consolidated from medial-temporal regions to neocortical areas (Squire and Alvarez 1995), a process linked to replay of neural activity during sleep (Foster and Wilson 2006; Lee and Wilson 2002; Skaggs and McNaughton 1996).

RS-fMRI can serve as a sensitive marker for many alterations in brain function. For example, changes in RS-fcMRI have been documented as a consequence of many diseases (Zhang and Raichle 2010), under anesthesia (Martuzzi et al. 2010), following extended training (Lewis et al. 2009), and during task performance (Fransson 2006). Although two recent reports have indicated that default-mode network integrity is diminished during sleep deprivation (De Havas et al. 2011; Sämann et al. 2010) and that the functional connectivity between the hippocampal formation and the rest of the default-mode network varies during different stages of sleep (Andrade et al. 2011), neither study examined morning-evening variation in brain functional organization while subjects maintained a typical sleep schedule.

We assessed differences in brain metabolism and RS-fcMRI between morning and evening in a total of 34 young adult subjects. Extensive quality control, including sleep assessments and EEG monitoring, was performed to ensure that these subjects were healthy.

EXPERIMENTAL PROCEDURES

We assessed differences in brain metabolism and RS-fcMRI between morning and evening in a total of 34 young adult subjects. Extensive quality control, including sleep assessments and EEG monitoring, was performed to ensure that these subjects were healthy.
typical sleepers. PET measurements were compared on both a global and regional basis. RS-fcMRI was analyzed using both a set of 36 a priori regions of interest, as well as an iterative whole brain search technique (IDEA).

Sleep assessments. Extensive measures were taken to ensure that our subjects were typical of the healthy population and did not suffer from severe sleep-related problems. In both the PET and fMRI studies, the Epworth Sleepiness Scale (ESS) and Pittsburgh Sleep Quality Index (PSQI) were used to evaluate subjects’ sleep habits. Information was also collected from a sleep diary that was kept over a 1-wk period (6–8 days), with results confirmed by simultaneously acquired actigraphy. Results are shown in Table 1.

Subjects in the PET study were also monitored with overnight EEG. In the week before the PET scans, each subject spent one night in our sleep laboratory to become acclimated to sleeping in an unfamiliar environment. In the night between the evening PET scan and the morning PET scan, subjects spent a second night in the sleep laboratory. EEG measurements were obtained and scored to quantify the amount of time spent in slow-wave sleep (SWS) and rapid eye movement (REM) sleep. These subjects were also monitored for apnea and hypopnea, evaluated using the apnea-hypopnea index (AHI).

Subjects fell within a normal range on most measures. One exception is the amount of time spent in SWS, which was about half the normal range. We attribute this to the laboratory environment; some subjects were probably not completely acclimated after a single night in the laboratory (Kingshott and Douglass 2000). These subjects were very likely normal sleepers in their own beds. We tested whether there was any relationship between individuals’ SWS and global PET measures, and found no significant correlations.

Two subjects (1 each from the PET and fMRI studies) fell outside the normal range on the ESS. Their scores were 11 and 12, respectively, whereas the normal range is 0–10 on a scale of 0–24. One of these subjects also scored outside the normal range on the PSQI (a score of 8 on a scale of 0–21, with a normal range of 0–5). These scores are not indicative of severe sleep disturbances. Because we wanted to sample the typical population, in which mild sleepiness is not uncommon, we chose to retain the data from these subjects in our analyses.

fMRI subjects. We obtained resting-state fMRI scans in 24 healthy young adults in the morning and the evening (15 male; ages 23–30 yr, mean age 25.9 yr). All subjects were right-handed native English speakers with no history of neurological or psychiatric disorder. All experiments were approved by the Institutional Review Board of Washington University Medical School, and each subject gave written informed consent. Handedness was assessed using the Edinburgh Handedness Inventory (Oldfield 1971). Subjects were scanned ~1 h after their usual wake time and 2 h before their usual bed time. The order of the morning and evening scans was counterbalanced across subjects. Heavy caffeine drinkers (those who consumed more than 1 cup of coffee or equivalent per day) were also excluded, and subjects were asked to abstain from caffeine for 24 h before the first scan.

We took a number of steps to ensure we included only healthy normal sleepers in the experiment. Subjects were queried about their sleep habits using the PSQI (Buysse et al. 1989) and the ESS (Johns 1991). For the week preceding the scans, subjects wore an actigraph, a wristwatch-like accelerometer that monitored movement (American Sleep Disorders Association 1995). Actigraphy data were analyzed to ensure that subjects maintained normal sleeping patterns before the scan. All subjects’ sleep patterns fell within a normal range (none exceeded 3SD from the mean on any measure), and no subjects were excluded on the basis of these data. The ESS showed no significant differences in sleepiness between morning and evening scans.

MRI acquisition. Structural and functional scans were performed with a 3-Tesla Siemens TRIO MRI. Structural data included a high-resolution, axial T1-weighted magnetization-prepared gradient-echo (MP-RAGE) scan and a high-resolution T2-weighted fast spin-echo scan for definitive atlas transformation. Functional data were acquired using a gradient-echo, echo-planar sequence sensitive to BOLD contrast (echo time, 25 ms; flip angle, 90°; 4 × 4 × 5-mm voxels; field of view, 256 × 256; repetition time, 2.08 s; bandwidth, 2,605 Hz). Two runs with 150 frames each were performed.

Resting-state functional connectivity analysis. Data were preprocessed using previously described techniques (Fox et al. 2009). This included a low-pass filter (0.1 Hz), spatial blur (Gaussian kernel, 6.2-mm full-width at half-maximum), and removal of nuisance regressors [signals from bilateral areas of white matter (cingulum; centered at ±32, −20, 30), lateral ventricles (±6, −10, 20), and whole brain; rotational and translational movement]. Average root-mean-squared movement across all subjects was 0.21 mm in the morning scans and 0.23 mm in the evening scans (P = 0.41). No subjects were excluded due to excessive movement. MR frames with excessive movement were excluded from consideration (those exhibiting movement-induced signal drop-off of more than 3 SD). Differences between morning and evening scans were assessed using an iterative data-driven algorithm (IDEA), as described below.

Iterative data-driven evolutionary algorithm. We employed a recently published technique to identify functional connectivity relationships that are reliably different between morning and night (Shannon et al. 2011). This algorithm searches throughout the brain for regions whose functional connectivity patterns can be used to predict independent variables (in this case, morning/evening status). The principal difference between IDEA and other strategies aimed at the same objective (to identify RS-fcMRI features that discriminate between groups or across continuous variables; Dosenbach et al. 2010; Krishnan et al. 2010; Supekar et al. 2008; Yang et al. 2010) is that the set of regions used for prediction is iteratively updated.

Our previous work with this algorithm identified functional connectivity effects associated with impulsivity in juveniles, using a continuous measure of impulsivity as the independent variable of interest. Here we adapted the algorithm to compare morning and evening resting-state scans within subjects (i.e., using a paired, discrete, independent variable).

Table 1. Subject sleep data

<table>
<thead>
<tr>
<th>Category</th>
<th>PET</th>
<th>FMR</th>
<th>Norms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Total sleep, h</td>
<td>7.26 ± 0.53</td>
<td>6.72–8.25</td>
<td>6.97 ± 0.48</td>
</tr>
<tr>
<td>Owl/lark (MEQ)</td>
<td>44.7 ± 4.8</td>
<td>35–56</td>
<td>50.0 ± 5.5</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>92.1 ± 2.2</td>
<td>87.3–95.4</td>
<td>88.7 ± 4.0</td>
</tr>
<tr>
<td>PSQI</td>
<td>3.70 ± 2.71</td>
<td>1–8</td>
<td>3.08 ± 1.35</td>
</tr>
<tr>
<td>ESS</td>
<td>6.10 ± 2.56</td>
<td>3–12</td>
<td>6.58 ± 2.69</td>
</tr>
<tr>
<td>SWS, %</td>
<td>8.2 ± 7.5</td>
<td>0–18.4</td>
<td></td>
</tr>
<tr>
<td>REM, %</td>
<td>20.0 ± 7.1</td>
<td>4.4–27.3</td>
<td></td>
</tr>
</tbody>
</table>

PET, positron emission tomography; fMRI, functional magnetic resonance imaging; MEQ, Morningness-Eveningness Questionnaire; PSQI, Pittsburgh Sleep Quality Index; ESS, Epsworth Sleepiness Scale; SWS, slow-wave sleep; REM, rapid eye movement sleep.
IDEA searches for regions whose functional connectivity is systematically altered in relation to the variable of interest. For example, a region would have strong predictive power if it very consistently showed high functional connectivity to the precuneus in the morning and low functional connectivity in the evening. Predictive power is computed quantitatively by creating a map of the paired $t$-statistic comparing a region’s functional connectivity in the morning and in the night (see step 1 below).

The key principal underlying IDEA is that correlation is a symmetrical relation. Any region with predictive power must express this predictive power through its functional connectivity with at least one other region. Therefore, we may identify new predictive regions by locating peaks in the functional connectivity/independent variable maps. We then repeat this procedure on the new regions, generating further regions in an iterative fashion. IDEA depends on three distinct procedures: 1) a procedure to quantitatively evaluate a region’s predictive power; 2) a procedure to identify new, potentially predictive regions by exploiting the symmetry property of correlation maps; and 3) a procedure to update the set of regions. These steps have been extensively described previously (Shannon et al. 2011). The algorithm is initialized by placing a number of regions into the testing set. Here, we used 36 “canonical” regions representing well-defined nodes in prominent brain networks, including the default-mode, dorsal-attention, executive-control, salience, visual, auditory, and motor networks (Raichle 2011). Critically, the entorhinal regions ultimately identified as highly predictive were not included in this initial set. Steps 1–3 are then iterated.

Predicting morning/evening status from functional connectivity.

The predictive ability of IDEA was tested using a leave-one-out cross-validation scheme. IDEA was run using data from 23 of the 24 subjects. Using the regions identified by IDEA, we generated a predictive model relating functional connectivity measurements to morning/evening status. We input data from the left-out subject into the model and evaluated its accuracy. Below we describe the predictive model using pseudocode:

For each left-out subject indexed by $j$ {
- Run IDEA.
- For each identified region indexed by $i$ {
  - Voxelwise compute the map $f(x,y,z)$, the paired $t$-test of morning/evening status on functional connectivity. Subject this map to cluster-based thresholding as described above, yielding a binary significance map $w_i$. Thus the value of a voxel in $w_i$ is 1 if the absolute value of the voxel’s $t$-statistic is greater than 3 and it is contiguous with at least 9 other voxels above threshold; otherwise it is 0.
  - Compute the morning-minus-evening difference map $\Delta_i$ for region $i$ using data from the left-in 23 subjects.
  - Compute the morning-minus-evening difference map $\delta_i$ for region $i$ using data from the left-out subject.
  - Evaluate the similarity between $\Delta_i$ and $\delta_i$ within significant voxels as follows:
    \[ Y_j = \sum \sum \int \Delta_i \delta w_i dV, \]
    where the integral is taken over the volume of the image. If $Y_j$ is positive, the model has correctly predicted the morning/evening status of the left-out subject’s two scans. If it is negative, it is incorrect.
}

Predictions were generated and tested for each left-out subject. Statistical significance was assessed using a chi-square test to compare the model’s performance to chance performance of 50% accuracy.

PET subjects. Ten subjects (5 female, ages 22–28 yr, mean age 22.5 yr) participated in the experiment. All subjects were right-handed native English speakers with no history of neurological or psychiatric disease. All experiments were approved by the Institutional Review Board of Washington University Medical School, and each subject gave written informed consent. Extensive prescreening and monitoring was performed to ensure that each subject was a healthy, typical sleeper who was fully rested on the day of the scans. Questionnaires (the PSQI, ESS, and Morningness-Eveningness Questionnaire) were administered to each subject. Each subject kept a sleep diary for 6–8 days before the scan, and diaries were confirmed with simultaneously acquired actigraphy. Subjects spent 2 nights in our sleep laboratory. The purpose of the first night was to acclimate the subjects to sleeping in an unfamiliar location; the second was to monitor their sleep cycles using EEG. Scans were performed at approximately 9:00 AM and 9:00 PM. All subjects were scanned first in the evening, second in the morning. During the intervening night, subjects slept in the sleep laboratory, with sleep stages monitored by continuously acquired EEG. Full results of these questionnaires and EEG overnight are available in Table 1.

PET acquisition. PET imaging was performed using a Siemens ECAT HRplus 962 scanner. Two PET scanning sessions were performed on each subject, one in the evening (study visit day +1) and one in the morning (study visit day +2). Before each PET session, an antecubital intravenous catheter was placed in the arm for tracer injection and blood sampling for glucose levels. Each PET session lasted ~3 h and consisted of seven PET scans and two transmission scans. The first transmission scan was acquired before any PET scans. Before the first PET scan, subjects were asked to inhale 40–75 mCi of radioactive carbon monoxide. Before the second PET scan, subjects were asked to inhale 40–75 mCi of radioactive oxygen. Before the third PET scan, subjects were injected with 25–50 mCi of radioactive water. The fourth to sixth PET scans were repetitions of the first to third scans. A second transmission scan was then acquired. For the seventh PET scan, a measurement of cerebral metabolic rate of glucose (CMRGluc) was initiated with an ~20-s bolus intravenous injection of 5 mCi of $[^{18}F]$fluorodeoxyglucose (FDG), immediately followed by a concomitant 60-min dynamic scan. Before the injection of FDG, a blood sample was withdrawn for measurement of glucose. A second blood sample was withdrawn ~30 min from the start of the FDG scan. All scans were obtained in the resting state; subjects were asked to lay quietly with their eyes open. Subject motion was minimized by fitting a thermoplastic mask over the face. Investigators spoke to subjects briefly between each scan and at 15-min intervals during the FDG scan to ensure the subjects had not fallen asleep.

PET analysis. The arterial input function (AIF) was estimated noninvasively by sampling activity in the carotid artery (Su et al. 2010, 2012). MP-RAGE and time-of-flight magnetic resonance angiography (TOF-MRA) images were coregistered to PET data and used to identify the petrous portion of the internal carotid artery. Regional activity for each PET image was extracted for a region of interest (ROI) representing the artery and a separate ROI immediately surrounding the arterial ROI. A two-tissue mixture model (artery and background) was assumed in the vicinity of the artery, and a linear system equation was solved to estimate the AIF.

\[
\begin{bmatrix}
aa \
ab
\end{bmatrix}
+ 
\begin{bmatrix}
Ca \
Cb
\end{bmatrix}
= 
\begin{bmatrix}
Ma \
Mb
\end{bmatrix}
\]

In the above equation, $aa$, $ab$, $ba$, and $bb$ are recovery and spillover coefficients defined on the basis of scanner point-spread function (customized to each radioactive tracer) and the artery/background segmentation. $Ma$ and $Mb$ are the measured PET signals in the arterial ROI and background ROI, respectively. The equation is solved for $Ca$ and $Cb$, the concentrations of radioactive tracer in the artery and background tissue, respectively.
This estimate of AIF was then used in conjunction with standard techniques to calculate absolute measures of cerebral blood flow (CBF), cerebral blood volume (CBV), cerebral metabolic rate of oxygen (CMRO₂) (Videen et al. 1987), and CMRGlú (Lucignani 1993). CBF was measured with a 40-s emission image (derived from a 120-s dynamic scan) after rapid injection of 25–50 mCi of [¹⁵O]water in saline (Raichle et al. 1983; Videen et al. 1987). CBV was measured with a 2.5-min emission scan beginning 2 min after brief inhalation of 40–75 mCi of [¹⁵O]-carbon monoxide in room air (Martin et al. 1987; Videen et al. 1987). CMRO₂ was measured with a 40-s emission scan (derived from a 120-s dynamic scan) after brief inhalation of 40–75 mCi of [¹⁵O]oxygen in room air (Mintun et al. 1984; Videen et al. 1987). Recirculating [¹⁵O]water of metabolism was measured and accounted for using the technique of Iida et al. (1993). Parametric images of net FDG trapping were created by application of the Patlak graphical method (Patlak and Blasberg 1985; Patlak et al. 1983). The noninvasively calculated AIF, derived from a linear-mixture model, was used in place of arterial blood sampling. Time-activity curves were then analyzed in the standard manner. Because calculation of FDG trapping was based on whole blood time-radioactivity data and not plasma activity, we calculated glucose concentration of whole blood as the mean of the plasma samples times \((1 - (0.30 \times \text{hematocrit}))\) (Dillon 1965). Implicit in these calculations is the assumption that there exists rapid equilibration between plasma and intracellular glucose. This has been demonstrated for human blood (Lefevre and McGinnis 1960; Murphy 1960).

Quantitatively accurate estimates of CMRGlú from FDG uptake require the lumped constant correction factor, since glucose and fluorodeoxyglucose are transported and metabolized to different degrees. There is controversy over the correct value for the lumped constant in normal brain. Measured values range from 0.52 to 0.886 and are to some extent dependent on the exact method used to compute net FDG uptake (Hassellbach et al. 1993, 1998; Reivich et al. 1985; Spence et al. 1998). The precise value of the lumped constant used for the experiments that we propose is not important, since it is a linear scaling factor applied to all the FDG trapping data and thus will not affect the statistical tests. We only assume that it is constant for the experimental conditions that we study. Since the lumped constant changes appreciably only in tumors or under conditions when glucose transport across the blood-brain barrier approaches the metabolic rate (such as ischemia and hypoglycemia), this is a reasonable assumption (Ball et al. 1988; Crane et al. 1981; Nakai et al. 1987; Spence et al. 1998). For these reasons we arbitrarily set the lumped constant to 1.0 in the calculation of regional and temporal changes in CMRGlú.

We have assessed regional differences in resting CMRO₂, CMRGlú, and aerobic glycolysis in a manner independent of whole brain quantitative measures (Vaishnavi et al. 2010). This strategy differs from that originally described in which absolute rates for CMRGlú and CMRO₂ were determined (Mintun et al. 1984; Videen et al. 1987). For each subject, measures of CBF, CBV, CMRO₂, and CMRGlú were aligned to each other and then to the subjects’ MRI scans. The realigned data were then transformed to atlas space using in-house software and scaled to a whole brain mean of 1 (local-to-global ratio as in Raichle et al. 2001). Our atlas-representative target image represents Talairach space as defined by Mazziotta et al. (1995). The ratio of oxygen to glucose consumption (OGI) was computed by a voxelwise division of CMRO₂ by CMRGlú to compute a local-to-global OGI. For comparison with traditional OGI measures, this ratio was scaled by 5.323 (Raichle et al. 1970).

**Regional PET measurements.** We believe that the PET measurements obtained in this experiment are likely to be of use to other investigators. Thus we have included a spreadsheet in Supplemental Materials that contains all measurements for each subject, calculated regionally for each Brodmann area. (Supplemental data for this article is available at the Journal of Neurophysiology website.)

### RESULTS

**Functional connectivity.** In the first experiment we used RS-fcMRI to investigate whether the cumulative effect of daily experiences is reflected in morning and evening changes in relationships among brain regions. We obtained resting-state fMRI scans in a group of 24 healthy young adults in the morning and the evening. The order of acquisition was counterbalanced such that half the subjects were scanned first in the morning and then in the evening of the same day, and half were scanned first in the evening and then in the morning of the next day. Subjects were healthy normal sleepers, as verified by sleep diaries, actigraphy, and a battery of questionnaires.

Increases in synaptic strength over wake, as posited by the synaptic homeostasis hypothesis, might well be expected to result in systematic changes in functional connectivity. As an initial test of this hypothesis we used 36 regions representing nodes of several major brain networks and calculated the correlation between each pair of regions during morning scans and evening scans (Fig. 1). No system-wide changes were observed. Intranetwork functional connectivity, as well as positive and negative correlations between networks, appeared unchanged. Furthermore, no individual pair of regions showed significant morning-evening differences after correction for multiple comparisons.

The 36 regions used in the above-described experiment represent important network nodes, but they cover only a fraction of the total brain volume. Therefore, we used a recently developed IDEA to search throughout the brain for regions exhibiting systematic differences in RS-fcMRI between morning and evening (Fig. 2). The operation of IDEA is described in detail in Shannon et al. (2011), as well as in **EXPERIMENTAL PROCEDURES.** Briefly, it begins with the same set of 36 regions described above. For each region, it compares the scans of each subject to search for areas whose functional connectivity systematically increases or decreases between morning and evening. These areas are themselves possible sites of interest, so the algorithm investigates them in the same way it investigated the original 36 regions. Each region is rated for the extent to which its RS-fcMRI changes between scans. This process continues iteratively until the algorithm converges on the highest rated regions, i.e., those whose RS-fcMRI is most significantly altered between morning and evening.

Our analysis identified two regions with strongly altered RS-fcMRI in the left and right medial temporal lobe (MTL; Fig. 3). It is noteworthy that these two regions are bilaterally symmetrical, even though IDEA provides no incentive or constraint to encourage symmetry. Functional connectivity patterns of the two regions, and their changes between morning and evening, were quite similar.

In the morning, the regions in left and right MTL were primarily connected locally with correlations in contralateral MTL, the surrounding area, and the temporal poles, as well as negative correlations in the striatum bilaterally (Fig. 4). In the evening, the regions of the left and right MTL displayed functional connectivity with neocortical areas, including the retrosplenial cortex, and nearby posterior cingulate and precuneus, bilateral angular and supramarginal gyri, and dorsolateral and dorsomedial prefrontal cortex. The MTL also exhibited in the evening positive correlations with the pontine tegmentum and with bilateral basal ganglia, as well as negative
correlations bilaterally with the anterior insula. The negative correlations with the striatum became positive correlations in the evening.

Strikingly, the neocortical regions to which MTL develops correlations in the evening are all closely involved in memory retrieval. Each consistently exhibits the retrieval success effect, in which activity in the regions is greater when subjects view previously experienced items compared with novel stimuli (Wagner et al. 2005). To confirm this overlap, we compared the map of morning/evening RS-fcMRI differences to a meta-analysis of fMRI memory studies investigating the retrieval success effect (Fig. 5). The overlap is remarkable, including medial and lateral parietal cortex as well as dorsolateral prefrontal and dorso-medial prefrontal regions. Moreover, we note that the anterior insula bilaterally are also often recruited during memory retrieval. Most consistently, their activity increases when subjects attempt to retrieve the source of a memory, successfully or unsuccessfully (Dobbins et al. 2003; Lundstrom et al. 2005).

Fig. 1. Functional connectivity is largely unchanged between morning and evening. Cross-correlation matrices between 36 brain regions are shown for morning and evening scans. No significant differences were observed following correction for multiple comparisons. The location and center-of-mass coordinates for each region are listed at top right. These locations are displayed visually on an inflated brain at bottom right.
Statistical validation. The iterative approach used in this study employs a very large number of comparisons to arrive at a final set of regions. It was therefore important to assess the reliability of the algorithm. To that end, we used leave-one-out cross-validation to test whether our method could predict the morning/evening status of scans from individuals whose data did not contribute to the generated model (Hastie et al. 2009). We repeated our analysis 24 times, each time leaving out a different subject’s morning and evening scans. We used the algorithm to make a prediction for which of the two left-out scans was performed in the evening and which was performed in the morning. This strategy addresses the issue of multiple comparisons: if the results that have been found on 23 subjects are indeed false positives due to multiple comparisons, then the morning/evening prediction from the left-out subject’s scan data will show only chance accuracy.

Our predictions fared far above chance. This operation correctly identified the morning and evening scans in 21 of 24 instances ($\chi^2 = 13.5; P < 0.001$). This, in combination with the bilateral symmetry of the MTL regions, as well as the identification of meaningful sets of memory-related regions, indicates that the algorithm uncovered real information about the brain. The algorithm was also quite consistent in its results. In all 24 instances, our algorithm identified the right MTL region as the region with the most altered RS-fcMRI; in 20 instances, it identified the left MTL region as the second most altered region.

One of the free parameters of IDEA is the number of regions contributing to the model. We evaluated the model’s accuracy using 1 to 10 regions (Fig. 6). Accuracy was highest with just 1 region at 87.5% and still significantly above chance with 2 regions at 70.8%. In each of the 24 leave-one-out instances, right entorhinal cortex was identified as the most predictive region; in 20 instances, left entorhinal cortex was the second most predictive region.

We have chosen to focus our discussion on entorhinal cortex because no other regions appear to contribute accuracy to the model. We discuss both left and right regions, rather than exclusively right, because we believe this reflects the underlying biology of the system. The model’s accuracy is lower with the left region included, but the anatomic location of the left region is closely symmetrical with the right, and it displays very similar patterns of functional connectivity. The symmetry of the predictive seeds as well as the associated morning vs. evening difference maps are unlikely to be a result of random chance.

Brain metabolism. To assess daily variation in brain metabolic costs, we obtained PET measurements in a second group of 10 subjects in the morning and in the evening. We verified with sleep diaries and questionnaires that subjects were healthy normal sleepers. Subjects slept 2 nights in the laboratory before the scans; their sleep patterns were monitored with EEG. We obtained scans using $^{15}$O$_2$, H$_2$O$^{18}$O, C$^{15}$O, and FDG. These allowed us to calculate several measures of brain metabolism, including CBF and CBV, CMRGlu and CMRO$_2$, oxygen-extraction fraction (OEF), and OGI (for a detailed review of these methods see Vaishnavi et al. 2010). To improve signal-to-noise ratio, each scan employing a $^{15}$O-labeled tracer was performed twice. With these measurements, we quantified global metabolic rates using a recently developed image-based algorithm to determine the arterial input function (Su et al. 2010, 2012). We also calculated maps of local-to-global ratios using techniques developed in our laboratory (Vaishnavi et al. 2010).

Somewhat to our surprise, the cost of brain function as measured by oxygen utilization (the most direct measurement of ATP use available with PET) and glucose consumption (a measure of both aerobic glycolysis and oxidative phosphorylation) was unchanged between morning and evening (Fig. 7A). Paired $t$-tests across the 10 subjects indicated that none of the measures were significantly different. Local-to-global ratios also revealed no difference between morning and evening. We performed voxelwise paired $t$-tests for CBF, CMRGlu, and CMRO$_2$. No significant differences were observed. Using standard confidence interval analysis, we can estimate with 95% confidence that the CBF change was between $-7\%$ and $+8\%$, the change in glucose uptake was between $-18\%$ and $+10\%$, and the change in oxygen consumption was between $-17\%$ and $+7\%$. These confidence intervals are far smaller than the consistent variations seen between different brain regions (Fig. 7, B–D). They are also far smaller than activity-related changes, which can range as high as a 68% increase in CBF (Ito et al. 2001).

Acquiring quantitative measurements of CMRO$_2$ and CMRGlu together in the same subjects permitted us to calculate global as well as regional levels of aerobic glycolysis (i.e., that fraction of glucose metabolized by the brain outside of oxidative phosphorylation). Because of the important role...
played by aerobic glycolysis in cellular biosynthesis and viability (Lunt and Vander Heiden 2011), this was a measurement of particular interest to us. Again, we found no differences between morning and evening in our subjects.

Finally, we tested whether the notable changes in the functional connectivity of left and right MTL and neocortex (experiment 1) were associated with any metabolic changes. We performed a post hoc analysis to test whether MTL or any of the frontal and parietal neocortical areas described above exhibited any changes in any of the PET measures of metabolism between morning and evening. No changes for any region for any measure were significant following multiple comparison correction.

DISCUSSION

Functional connectivity. Our data reveal that medial temporal lobe (MTL) functional connectivity changes dramatically over a normal daily period of wake. These bilateral changes in MTL functional connectivity are likely related, at least in part, to memory function.

MTL has been extensively characterized as critical in the storage and retrieval of memories (Squire et al. 2004). The prefrontal and posterior parietal regions have received less attention, but they are also associated with memory retrieval (Konishi et al. 2000). fMRI studies of memory retrieval have consistently found that these regions show greater activity in response to previously experienced items compared with novel stimuli (Konishi et al. 2000; Shannon and Buckner 2004; Vann et al. 2009). Some subregions show greater activity for recollective retrieval compared with familiarity-based recognition (Donaldson and Buckner 1999).

Moreover, evidence indicates that MTL’s functional connectivity with neocortical memory regions is reduced during sleep. In a comparison of evening wakefulness, stage 1 sleep, stage 2
sleep, and SWS, MTL functional connectivity with other default-mode network regions was reduced as subjects transitioned to deeper sleep stages (Andrade et al. 2011). In addition, a psychophysical interaction (PPI) analysis revealed that this functional connectivity was stronger during sleep spindles. Spindles are believed to represent an electrical signature of mechanisms involved in memory consolidation (Gais et al. 2002; Schabus et al. 2004). This sleep-related reduction in functional connectivity dovetails with results from the PET literature, which indicate that MTL blood flow increases during...
functional connectivity between MTL, the cerebral cortex, and the striatum between morning and evening may well be facilitated by activity within brain stem nuclei such as the locus coeruleus.

Our results, in combination with those of Andrade et al. (2011), give us a picture of a full day of MTL functional connectivity. MTL is primarily locally correlated in the morning but exhibits neocortical, striatal, and brain stem correlations in the evening. As sleep progresses, the functional connectivity returns to a morning-like pattern. One exciting, though speculative, possibility is that the evening MTL functional connectivity arises as subjects accumulate experiences and memories throughout the day and recedes as those memories are consolidated during sleep.

This finding contributes to a small but growing body of evidence indicating that functional connectivity relationships are not constant but vary over the course of minutes (Chang and Glover 2010) and hours (Park et al. 2012), even in the absence of experimental intervention. Whereas previous reports characterize mainly stochastic variability (i.e., variation that occurs without apparent relationship to other variables), the present report documents a systematic change between morning and evening. Interestingly, Park et al. (2012) anticipated our results to some extent, identifying a hippocampal network as showing “high dynamicity,” although they did not investigate morning-evening effects.

Both stochastic and systematic variability have practical consequences with regard to the study of functional connectivity. Investigators using functional connectivity as a clue to structural connectivity should be aware that their results may vary depending on the time of day a scan is performed. For example, functional connectivity between posterior parietal and medial temporal regions has been reported previously (Greicius et al. 2003; Vincent et al. 2006). If the scans for that study had been obtained exclusively in the morning, the data may have indicated a different conclusion.

Finally, sleep disturbances are a prominent feature of many disorders, including depression and Alzheimer’s disease. If the circadian changes in MTL functional connectivity observed here are biomarkers of healthy sleep, they may be useful in the treatment of these disorders.
furthering our understanding the neurobiological significance of the sleep disturbances in these diseases.

Metabolism. Synaptic activity accounts for the largest fraction of the brain’s enormous energy budget (for a review see Raichle and Mintun 2006). Because functional connectivity likely depends on synaptic efficacy and number, changes in functional connectivity and, hence, synaptic efficacy and/or number during wake of the type we found in experiment 1 might be expected to have an effect on brain metabolism. Somewhat surprisingly, we found no evidence for an increase in global or regional metabolism between morning and evening in a group of 10 healthy young adults in whom we documented normal sleep-wake cycles during the week preceding our study as well as the night between our measurements of brain circulation and metabolism. To put our findings into perspective, it is important to consider two things: first, the work of others, and second, the scope of metabolism, which includes not only the generation of energy but also biosynthesis. First, we turn to a brief summary of extant work on brain metabolism related to sleep and wake.

Beginning with the first quantitative study of brain circulation and metabolism prior to sleep in humans (Mangold et al. 1955),1 there have been a total of six additional studies in humans that we know of (Boyle et al. 1994; Braun et al. 1997; Buchsbaum et al. 1989; Madsen et al. 1991a, 1991b; Thomas et al. 2000), one in monkeys (Kennedy et al. 1982), one in cats (Reivich et al. 1968), and one in mice (Vyazovskiy et al. 2008).

We will restrict our comments to the quantitative studies in humans because they are most relevant to our results. Only one study has been performed in humans before and after a night’s sleep. Braun et al. (1997) measured regional and global cerebral blood flow with PET before, during, and after sleep in a group of sleep-deprived but otherwise normal young adults. In these individuals blood flow was 54 ml·g⁻¹min⁻¹ in the evening before sleep, a value significantly higher (17%) than that obtained the following morning. However, it should be noted that the value they obtained in the evening was within the range of normal values obtained in humans without regard to the time of day (e.g., see Kety and Schmidt 1948; Powers et al. 2007; Raichle et al. 1970). The authors suggested this difference could be due to increases in blood flow due to sleep deprivation or a decrease brought about by intervening “recovery sleep” (or both). Our data in well-rested subjects (49 ml·g⁻¹min⁻¹) would suggest that sleep deprivation did not have a significant influence on the evening blood flow.

Three other studies in humans prior to sleep (Boyle et al. 1994; Madsen et al. 1991b, 1991c) support the above conclusion. As in the study by Braun et al. (1997), the subjects in the study by Boyle et al. had been sleep deprived at the time of the evening measurement, but sleep deprivation was not employed in the two studies by Madsen and colleagues. Boyle et al. observed a presleep blood flow of 50 ml·g⁻¹min⁻¹. In the two studies by Madsen and colleagues in non-sleep-deprived subjects (1991b, 1991c), the presleep values were 58 and 57, respectively. Thus, combining these results with our own data, it would appear that sleep deprivation does not explain the blood flow difference observed in the experiment by Braun et al. (1997). Further work is needed to explain the
low value of blood flow in the morning observed by these investigators.

Our measurements of oxygen consumption and glucose utilization, which show identical values for morning and evening, are within the range of evening values observed in those studies in which metabolism was measured before sleep (Boyle et al. 1994; Buchsbaum et al. 1989; Madsen et al. 1991b, 1991c; Mangold et al. 1955; Thomas et al. 2000). It is important to emphasize that ours is the only study in which metabolism was measured both before and after sleep and revealed no changes globally or locally as the result of wake.

Before dismissing the role of brain metabolism in the synaptic events of wake (clearly demonstrated in our experiment 1), it is important to consider a broader vision of the role of metabolism in brain function. Traditionally, it has been tacitly assumed by most that the consumption of glucose and oxygen represents the brain’s need for energy without consideration of possible additional roles. However, we know that glucose consumption exceeds that needed for oxidative phosphorylation by about 15% in the resting adult human (Powers et al. 2007; Raichle et al. 1970). This has traditionally been referred to as aerobic glycolysis (i.e., glucose consumption occurring in excess of that needed for oxidative phosphorylation in the presence of adequate tissue oxygenation). Furthermore, aerobic glycolysis is nonuniformly distributed in the brain, being high in the brain’s default-mode network and prefrontal control systems and reduced in the cerebellum and medial temporal lobes (Vaishnavi SN, Vlassenko AG, Shannon BJ, Larson-Prior L, Snyder AZ, unpublished observations). Finally, aerobic glycolysis represents a far larger share of glucose utilization in the developing human brain (Altman et al. 1993; Chugani et al. 1987; Powers et al. 1998), which is consistent with the important role of aerobic glycolysis in biosynthesis (Lunt and Vander Heiden 2011).

Importantly, aerobic glycolysis may be involved in synaptic events associated with memory and learning. In a remarkable study by Madsen et al. (1995), they were able to show that aerobic glycolysis persists for close to an hour following the performance of a difficult cognitive task without any evidence of an increase in oxygen consumption or blood flow. This observation, coupled with the metabolic measurements we report herein (i.e., no change in aerobic glycolysis either globally or regionally between morning and evening), suggests that some of the metabolic consequences of synaptic growth and strengthening may be time limited within the period of wake. Obviously, more work needs to be done that emphasizes a more comprehensive view of glucose metabolism. The idea of “local sleep” during wake (Vyazovskiy et al. 2011) should also be considered.

GRANTS
This work was supported by National Institutes of Health Grants NS 006833, NS 048056, and MH 077967 and a grant from the James S. McDonnell Foundation.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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J Neurophysiol • doi:10.1152/jn.00651.2012 • www.jn.org

Downloaded from http://jn.physiology.org/ by 10.220.32.247 on October 8, 2016
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