Impact of time-of-day on brain morphometric measures derived from T₁-weighted Magnetic Resonance Imaging

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ABSTRACT

Measures of brain morphometry derived from T₁-weighted (T₁W) images are widely used to elucidate the relation between brain structure and function. However, the computation of T₁W morphometric measures can be confounded by subject-related factors such as head motion and level of hydration. A recent study reported subtle yet significant changes in brain volume from morning to evening in a large group of patient populations as well as in healthy elderly individuals. In addition, there is a growing recognition that factors such as circadian rhythm can impact MRI measures of brain function and structure. Here, we provide a comprehensive assessment of the impact of time-of-day (TOD) on widely used measures of brain morphometry in a group of 19 healthy young adults. Our results show that (a) even in a small group of healthy adult volunteers, a highly significant reduction in apparent brain volume, from morning to evening, could be detected; (b) the apparent volume of all three major tissue compartments—gray matter, white matter, and cerebrospinal fluid—were influenced by TOD, and the magnitude of the TOD effect varied across the tissue compartments; (c) measures of cortical thickness, cortical surface area, and gray matter density computed with widely used neuroimaging software suites (i.e., FreeSurfer, FSL-VBM) were all affected by TOD, while other measures, such as curvature indices and sulcal depth, were not; and (d) the effect of TOD appeared to have a greater impact on morphometric measures of the frontal and temporal lobe than on other major lobes of the brain. Our results suggest that the TOD effect is a physiological phenomenon and that controlling for the effect of TOD is crucial for proper interpretation of apparent structural differences measured with T₁W morphometry.

INTRODUCTION

In clinical neuroimaging, T₁-weighted (T₁W) images are one of the most powerful diagnostic tools afforded by magnetic resonance imaging (MRI). The striking contrast between gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) in T₁W images makes it possible for the trained eye to detect pathological abnormalities in the living human brain. Incremental advancements in the optimization of the T₁W pulse sequence, in terms of higher spatial resolution and contrast-to-noise levels, as well as the development of sophisticated postprocessing software packages, have led to widespread adoption of this MRI technique by the neuroimaging research community. Importantly, with images from a 3D magnetization-prepared rapid gradient-echo (MPRAGE) scan (Mugler and Brookeman, 1990) that can be acquired at a spatial resolution of 1 mm (isotropic) in as little as five minutes, software packages such as FreeSurfer (Dale et al., 1999; Fischl, 2012; Fischl et al., 1999) can automatically derive various types of morphometric measurements of the brain that are thought to reflect properties such as cortical thickness, ventricular volume, sulcal depth, and indices of brain curvature. These advancements, along with findings from studies that have related some of these morphometric measures to histological data (Cardinale et al., 2014; Rosas et al., 2002), have rendered a quantitative veneer to this MRI technique. Thus, T₁W morphometry is being widely used to investigate training-related plasticity (for a review see, Thomas and Baker, 2013), correlations between structural differences in brain morphometry and behavioral measures (For a review, see Kanai and Rees, 2011), brain development (Giedd et al., 1999), aging (Salat et al., 2004), and a wide range of brain disorders such as Alzheimer's disease (Frisoni et al., 2010) and autism spectrum disorders (Chen et al., 2011).

Given the popularity of T₁W morphometry, it is crucial to identify the factors that can affect the MRI signal and, in turn, influence the computation of various measures of brain morphology. In the MRI environment, such confounding factors can be broadly categorized into those originating either from the MRI hardware/software or the subject. MRI hardware/software factors such as field

strength of the magnet, type of multichannel receiver coils, spatial distribution of the receiver coils, acceleration, and coolant temperature can bias the signal in the reconstructed T₁W images in subtle yet significant ways (Han et al., 2006). The effect of some of these magnet-related factors (e.g., field strength, receiver coils) may not be important practically as they typically remain constant within an experimental study. However, it is crucial to control for confounds introduced by the subjects.

For example, level of hydration of individual subjects has been shown to impact volumetric measurements of brain structures. Specifically, acute dehydration has been found to result in an increase in the apparent volume of the ventricles (Dickson et al., 2005; Kempton et al., 2009) with no significant change in total brain volume (but see, Duning et al., 2005). Similarly, using voxel-based morphometry (VBM), Streitbürger et al., (2012) found that dehydration results in a reduction in the volume of GM and WM in specific brain regions, along with an increase in ventricular volume. In contrast, hyperhydration was found to increase white matter volume significantly (Streitbürger et al., 2012). Further, head motion during acquisition of the T₁W images has been demonstrated to bias measurements of the volume and thickness of cortical gray matter (Reuter et al., 2015).

A growing number of studies have reported diurnal changes in MRI based measures of brain structure derived from T₁W images. For example, Maclaren et al., (2014) found that one among three subjects who were scanned N times across 31 days during different hours of the day, showed a significant increase in ventricular volume that was correlated with the time of day. In a much larger group of healthy volunteers (N = 404) Miller et al., (2015) found that seasonal differences in the length of a day accounted for differences in the volume of the hippocampus. A recent retrospective analysis of an even larger corpus of T₁W data revealed a significant time-of-day (TOD) effect in the measurement of brain volume (Nakamura et al., 2015). Specifically, this study reported a reduction in brain volume from morning to evening that was observed across patient populations as well as healthy elderly individuals.

TOD-related changes have also been observed using MRI methods other than T₁W morphometry. For example, using diffusion tensor MRI (DTI) Jiang et al., (2014) found significant changes in various DTI measures of tissue microstructure from morning to evening. Likewise, diurnal variations in glucose metabolism has been reported using positron emission tomography (PET) (Buysse et al., 2004). Finally, fluctuations in regional cerebral blood flow (rCBF) and functional connectivity have been detected using arterial spin labeling (ASL) and resting state functional MRI (rs-fMRI) respectively and the variation in these measures correlated with the participant's cortisol levels suggesting the role of circadian rhythms (Hodkinson et al., 2014).

While these studies provide converging evidence for the effect of TOD on MRI measures of brain structure and function, there remain several open questions regarding the impact of TOD on measures of brain morphometry derived from T₁W images. First, whereas Nakamura et al. reported a decrease in brain volume ranging from -0.09% to -0.221% of intracranial volume (ICV) from morning to evening, this estimate was drawn from an extraordinary sample (N = 1589; 9383 MRI scans) comprising patients with neurological disorders and healthy elderly controls. Thus, whether TOD is a significant confound in typical T₁W morphometry studies involving relatively smaller groups of young healthy individuals is unknown. Second, although the TOD effect has so far been described as a gross change in brain volume (Nakamura et al., 2015), or specific structures like the hippocampus (Miller et al., 2015) or the lateral ventricles (Maclaren et al., 2014), it is unclear exactly how TOD may differentially affect the apparent volume of the three main tissue compartments (i.e., gray matter, white matter, and CSF), and whether certain brain regions are disproportionately impacted. Third, whether the apparent change in whole-brain volume also impacts surface-based T₁W morphometry measures (i.e., cortical thickness, surface area, curvature, and sulcal depth) as well as VBM measures has not been determined. Finally, whether the effect of TOD is indeed a concern in longitudinal studies involving experimental manipulations, such as behavioral

training, has not been directly assessed. In the present study, we addressed these questions systematically in a group of healthy young adults.

MATERIALS AND METHODS

Research Participants

Twenty-one adult volunteers participated in the present study as part of a larger project that required multiple visits. However, we report data from 19 participants (mean age: 25.8 years; range: 20–38, 10 female) because not enough data were acquired from two subjects due to participant unavailability and pulse sequence malfunction. All participants were screened for any history of neurological and psychiatric disorders, as well as drug and alcohol abuse, in a comprehensive interview and determined to be healthy. The participants were also determined to be right-hand dominant based on the Edinburgh handedness inventory (Oldfield, 1971). All participants gave written informed consent. As per the policy of the National Institutes of Health (NIH) clinical center, female participants were required to take a standard pregnancy test immediately before the morning scanning session each day. The consent and protocol used for this study were approved by the NIH Institutional Review Board (Protocol 93-M-0170, NCT00001360).

Study Design

As illustrated in Figure 1, each participant was scanned across four independent, consecutive visits, with one week between visits. During each visit, participants were scanned from 10AM to 12PM (AM session) and from 2PM to 4PM (PM session), with a two-hour rest period in between sessions. During Visits 1 and 4, participants were not given any specific instructions during the rest period, and most participants used the time as a lunch break. However, during Visits 2 and 3, participants were given a short lunch break in addition to training on specific computer-based visuo-motor tasks as part of a study investigating training-dependent changes in the brain. In addition, between Visits 3 and 4, all participants were given 45 minutes of training per day in a visuo-motor task for approximately four to five consecutive days. In the present study the main focus

is on the effect of TOD, independent of any behavioral interventions; therefore, full details of the training paradigms are not provided here.

INSERT FIGURE 1 APPROXIMATELY HERE

The timing of the scans was strictly observed for all participants, although due to administrative and technical reasons there were some minor deviations in the exact timing of the data acquisition during a small number of scanning sessions. Given that Visits 1 and 4 did not involve any intervention between the AM and PM sessions, we used the data from these sessions as the primary dataset to investigate the effect of TOD for the purposes of this study. However, since one of our objectives was to directly assess the effects of TOD in the context of measuring structural changes in the brain following training, we used the data from Visits 3 and 4 as a secondary dataset.

MRI Acquisition

Participants were scanned on a 3 Tesla General Electric MR750 scanner with a GE 32-channel head coil. In addition to several other MRI sequences, two separate T₁W MPRAGE volumes were acquired (Scan 1 and Scan 2) during each session. Each T₁W MPRAGE scan was acquired with a standard imaging protocol, which is widely used by investigators at NIH for structural MRI studies [scan parameters: accelerated sagittal 3D MPRAGE, 25 cm FOV, 256 x 256 matrix slice, 1 mm slice thickness; TR: 7 ms; TE: 3.42 ms; flip angle: 7 deg, SENSE acceleration factor (ASSET): 2; acquisition time: 5 min 23 s]. The two sets of MPRAGE volumes were typically acquired after the acquisition of images for a 3-plane localizer, ASSET calibration, and T₂-weighted scan. Immediately after each acquisition, the T₁W volume was subjected to a qualitative inspection, and the volume was reacquired within the same session if the quality of the images was found to be inadequate. In total, the primary dataset comprised 152 T₁W scans acquired from 19 healthy volunteers. A single scan in the secondary dataset was deemed inadequate for analysis during the qualitative inspection. Therefore, the secondary dataset comprised 151 T₁W scans, acquired from the same participants.

Processing of T₁W images

We used the methods employed by two widely used software packages: FreeSurfer (<http://freesurfer.net/fswiki>) and FSL-VBM (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM>). We adopted these methods primarily to provide meaningful estimates of the TOD effect to the large community of users of these software packages.

FreeSurfer Analysis: The FreeSurfer image analysis suite (surfer.nmr.mgh.harvard.edu, Version 5.3) was used for computing volumetric and various surface-based morphometric measures of the brain. First, each participant's T₁W structural images were processed automatically as a cross-sectional dataset by using the "recon-all" command with default options (Dale et al., 1999; Fischl et al., 1999). Upon completion, a subject-specific template was created for each participant by using the automated FreeSurfer longitudinal stream, because this approach has been shown to significantly reduce within-subject variability and increase statistical power (Reuter et al., 2012). Finally, each participant's images were reprocessed with information from their subject-specific template (Reuter and Fischl, 2011). These final reprocessed images were used to derive all volumetric and surface-based metrics. Because the type and version of the operating system have been shown to impact measurements computed by FreeSurfer (Gronenschild et al., 2012), all images for this study were processed on computers running a standard version of Linux (Red Hat Enterprise v5.3).

Volumetric measurements were derived by using FreeSurfer's automatic segmentation procedure (Fischl et al., 2002). This procedure segments the brain into white matter, gray matter, and CSF by using the differences in the intensity profile of each voxel. The classification of voxels into various tissue types was further refined by using spatial information from a probabilistic atlas of brain anatomy, and finally volumetric statistics of different brain regions were automatically generated. To correct for differences in subjects' skull size, all volumetric measurements were divided by subjects' ICV, which was

automatically estimated by FreeSurfer with a procedure described by Buckner et al., (2004). Therefore, the volumetric measurements reported here are expressed as a percentage of ICV. Finally, volumetric regions that appear in both hemispheres were averaged to create a single volumetric region for statistical testing. Thus, FreeSurfer was used to compute various global measures of tissue volume such as Total Brain Volume (represented in FreeSurfer as the sum of BrainSegNotVent and brain stem), Total Gray Matter Volume (represented in FreeSurfer as TotalGray), Total White Matter Volume (sum of cortical and cerebellar white matter), Total CSF Volume (sum of all ventricle volumes, choroid-Plexus, and sulcal CSF), as well as volumetric measures of specific brain regions and subcortical structures (for more details see: surfer.nmr.mgh.harvard.edu/fswiki/MorphometryStats).

The surface-based measurements were derived by using FreeSurfer's automatic reconstruction procedure. This procedure creates white- and gray-matter surface models of the cortex, composed of vertices (Fischl and Dale, 2000), from which several surface-based measures—such as cortical thickness, surface area, sulcal depth, gyral height—as well as indices of brain curvature (i.e., fold index, curvature index, Gaussian curvature, and mean curvature) can be automatically extracted. Surface-based measurements that showed a highly significant effect of TOD across the entire cortex were further investigated to determine the distribution of the effect across different cortical regions. This analysis was performed using an automatic parcellation procedure (Fischl et al., 2004), which parcellates the cortex into 68 distinct regions (34 per hemisphere) based on the Desikan-Killiany atlas (Desikan et al., 2006) (for details see surfer.nmr.mgh.harvard.edu/fswiki/CorticalParcellation). The average morphometric measures from each of these 68 cortical regions were then tested for a TOD effect, and the results were plotted on a standard cortical surface after correction for multiple comparison by using the false discovery rate (FDR) method. To further test for the specificity of the TOD bias in certain brain regions, these 68 cortical regions were then regrouped into six major cortical areas (i.e., frontal, occipital, temporal, parietal, cingulate, insula) according to FreeSurfer's

documentation (for details, again see surfer.nmr.mgh.harvard.edu/fswiki/CorticalParcellation). All visualizations of the surface-based maps were performed with the Surface Mapping (SUMA) toolbox from AFNI (Saad et al., 2004)(afni.nimh.nih.gov/afni/suma).

Voxel-Based Morphometry. VBM uses spatial normalization and tissue classification methods to generate whole-brain gray matter maps of varying intensity fields that are presumed to approximate the density or concentration of local gray matter (Ashburner and Friston, 2000). These gray matter maps are then modulated by the Jacobian of the nonlinear transformation to produce maps of grey matter density (Ashburner and Friston, 2001). VBM was originally designed to detect cortical atrophy in patient populations, but it has been increasingly adopted for exploring differences in brain morphometry in the context of experimental interventions (Thomas and Baker, 2013). Although VBM is similar to FreeSurfer in that it uses the tissue contrast in T₁W images to create probability maps of GM, WM, and CSF, the results, in terms of group differences, do not typically converge (Bermudez et al., 2009). In fact, inconsistencies even in the results generated from different implementations of VBM have been demonstrated (Thomas et al., 2009). In the present study, we used the VBM implementation by FSL (fmrib.ox.ac.uk/fsl/fslvbm/, Version 1.0) to test whether the TOD effect is independent of the processing pipeline. Specifically, all T₁W images were corrected for image inhomogeneities with FAST (Zhang et al., 2001) and then processed through a modified VBM-FSL pipeline designed for longitudinal analysis (Douaud et al., 2009; Thomas et al., 2009). Resulting GM density images were smoothed with an isotropic Gaussian kernel (sigma = 3.5 mm, analogous to an 8-mm FWHM). Finally, a mixed-effects analysis was performed on all voxels with a minimal modulated GM density of 0.2 (across all images).

Measuring Subject Motion

Real-time estimates of subject head motion during acquisition of T₁W images are ideally measured by using volumetric navigator images (Reuter et al., 2015;

Tisdall et al., 2012). In the present study, although head motion could not be measured in real time, alternative measures of subject head motion were derived. Specifically, given that diffusion-weighted MRI (DWI) data were also acquired during each session along with the T₁W images, the motion-correction transformations computed from preprocessing of the DWI data can be considered as an indirect measure of a participant's ability to stay still during structural MRI scans. We therefore processed the DWI data with TORTOISE (Pierpaoli et al., 2010), which uses a mutual-information-based registration algorithm to align in 3D the diffusion-weighted volume to a non-diffusion-weighted structural volume (Rohde et al., 2004). The displacement computed along X, Y, and Z directions following registration served as a proxy measure of each participant's intrinsic head motion during a scanning session.

Statistical Analysis

TOD was determined from the acquisition time in the header of the Digital Imaging and Communications in Medicine (DICOM) file. To quantify the relationship between each brain morphometry measure and TOD, we applied the linear mixed effect (LME) model using the lme4 package in R (Bates et al., 2014).

For each measure of brain morphometry computed from the primary dataset (i.e., Visits 1 and 4) as well as Total Brain Volume computed from the secondary dataset, the model used consisted of fixed effects for both intercept and slope and a random effect to account for individual variations. The model equation is shown in Eq. (1).

$$\text{measure}_{i,j} = B_0 + B_1(\text{TOD}) + b_j + e_{i,j} \quad (1)$$

where

- $\text{Measure}_{i,j}$ is the T₁W structural measurement value for subject i at time point j ;
- $[\text{TOD}]$ is the time-of-day of the T₁W scan scaled from 0 to 1 for 24 h;
- $B_{0,1}$ are the fixed effect coefficients: B_0 is the intercept, B_1 is the linear slope for $[\text{TOD}]$;

- b_j is the random effect coefficient for subject i and assumed to have zero mean and a constant variance;
- $e_{i,j}$ is the error for subject i at time point j .

The subject-specific effect (i.e., the random effect) in our mixed-effect model estimates the correlation between measurements taken from the same subject. and accounts for multiple measurements taken from the same subject. The fixed effect of TOD was added to the random effect of the subject to estimate a prediction for each value of the T₁W structural measure being modeled. The residual error between the prediction and actual measurement was calculated and used for significance testing and, wherever applicable, p -values were adjusted to correct for multiple comparisons by using the FDR procedure.

Three additional models were tested by using the primary dataset to assess whether a significant TOD-by-gender, TOD-by-age, or TOD-by-head-motion interaction effect was present with respect to total brain volume. As shown in Eq. (2), these models included random intercepts for individual subjects; fixed effects for TOD; and a covariate that was either gender, age, or head motion, as well as the interaction between TOD and the respective covariates.

$$\begin{aligned} \text{measure}_{i,j} = & B_0 + B_1(\text{TOD}) + B_2(\text{covariate}) + \\ & B_3(\text{TOD} * \text{Covariate}) + b_j + e_{i,j} \end{aligned} \quad (2)$$

The covariates are defined as follows:

- (Gender) is a binary variable that multiplies 1 or 0 for Male or Female;
- (Age) is the age in years of the subject during Visit 1;
- (HeadMotion) is the measured propensity for head motion during a structural scan for subject i at time point j ;
- (TOD*Covariate) is the interaction between the covariate term and the TOD of the T₁W scan;
- $B_{2,3}$ are the fixed effect coefficients: B_2 is the linear slope for the (Covariate); B_3 is the coefficient for the interaction term (TOD*Covariate).

As noted earlier, the volumetric measure computed for each tissue compartment is expressed as a percentage of ICV. To estimate the change in tissue volume from morning to evening for a specific tissue compartment, we used the following formula:

$$B_1(\text{TOD}(\text{TissueType}))/\text{Total Volume (TissueType) relative to ICV}$$

where TissueType could be CSF, GM, or WM

Surface-Based Major Region Analysis

To test whether the TOD effect biases the measurement of apparent cortical thickness in specific brain regions, we grouped the 68 parcellated cortical regions into six major lobes or regions (i.e., frontal, temporal, occipital, parietal, cingulate, and insula) based on FreeSurfer's lobe-mapping instructions (for more details see surfer.nmr.mgh.harvard.edu/fswiki/CorticalParcellation). We then calculated the difference in cortical thickness between each participant's average thickness during morning and afternoon scans for each major region. Next we ran a one-way ANOVA using region as a factor with six levels. Post hoc paired *t*-tests were used to determine if any specific regions were significantly more impacted than any of the others.

Correlation Analysis

To test for correlations between the TOD-related volume change in the three main tissue compartments (GM, WM, CSF), we first calculated for each subject the difference in volume in each tissue compartment between the first morning and afternoon scans as well as the second morning and afternoon scans for the primary dataset. We then used the `cor.test` function in R package, to assess correlations between CSF change versus GM change, CSF change versus WM change, and WM change versus GM change. This function estimates the Pearson's product moment correlation coefficient *r* and *p*-values. This function computes a test statistic based on *r*, which follows a *t*-distribution with the number of degrees of freedom (*df*) equal to `length(n)-2`, where *n* is the number of

scans in our analysis. Finally, we used the function, `paired.r` in the R package to test the difference between pairs of correlated correlations.

RESULTS

FreeSurfer-Based Analysis: We first tested for a TOD effect on whole-brain volume based on the volume- and surface-based measures derived using FreeSurfer. The fixed effect of TOD on total brain volume (i.e., GMV + WMV + Brain Stem + Vessel – CSF volume) was highly significant [–2.289% of ICV per 24 h, standard error (SE) = .3263, $p < 1.0 \times 10^{-10}$] and confirms the findings from Nakumura et al. 2015 in a much smaller and younger sample (Figure 2a). Our results suggest that in a group of healthy adult volunteers, total brain volume reduces by 0.1% of ICV per hour, which amounts to an average decrease of approximately 1471 mm³, (SE = 211) of brain volume per hour from morning to afternoon [see Supplementary Figure 1 for subject-specific decrease in total brain volume (TBV)].

INSERT FIGURE 2 APPROXIMATELY HERE

Testing for any potential interactions between TOD and factors such as gender, age, and subject head motion as measured from the diffusion MRI scans did not reveal any significant interactions [Gender: –0.104 (SE = 0.325), $p = .75$; Age: –0.0153 (SE = 0.055), $p = .78$; Head Motion –0.0153 (SE = 0.055), $p = .78$]. These results demonstrate the effect of TOD on brain volume estimated from the primary dataset in which subjects were not given any specific tasks in between the AM and PM scans. Next, we directly tested whether the TOD effect is a significant confound in the context of a behavioral training experiment, in which the same participants were given training in a visuo-motor paradigm between the morning and afternoon scan sessions. Repeating the same analysis on the secondary data set (Visits 2 and 3) again revealed a significant main effect of TOD (–0.8450% of ICV per 24 h, SE = 0.3767, $p = .026$), although, compared to the TOD effect observed in the primary data set, behavioral training between the scan sessions appears to attenuate the magnitude of the TOD effect (see Supplementary Figure 2).

These results are, in general, consistent with those reported by Nakamura and colleagues (2015). However, given that our primary objective was to undertake a comprehensive exploration of the impact of TOD, we next examined whether the TOD effect differentially impacts the three major brain tissue compartments. As shown in Figures 2b and 2c, with TOD as a fixed effect, the LME analysis revealed a significant decrease in total gray matter volume (GMV) (–1.858% of ICV per 24 h, (SE) = .3053, $p < 1.0 \times 10^{-8}$) as well as total white matter volume (WMV) (–.487% of ICV per 24 h, (SE) = .1416, $p < .0007$), and a significant increase (Figure 2d) in total CSF volume (0.16942% of ICV per 24 h, (SE) = 0.02277, $p < 1 \times 10^{-11}$) from morning to afternoon. While the TOD effect does impact all tissue types, our estimation of the magnitude of change in the volume of a specific tissue compartment indicates that the TOD effect appears to be the greatest on CSF volume (0.222%), followed by GMV (–0.047%) and WMV (–0.016%), respectively. This finding suggests a disproportionate impact on the CSF and GM compartments compared with WM.

To explore the relation between the decrease in volume in GM and WM and the increase in CSF volume, we performed the Pearson's product-moment correlation test. As shown in Figure 3, the analysis revealed a robust negative correlation between the TOD-related decrease in GMV and the increase in CSF volume ($r = -0.527$, $p < 1.011 \times 10^{-6}$). The decrease in WMV was also found to negatively correlate with the increase in CSF volume, although compared to the correlation with GMV, the association appears less robust ($r = -0.293$, $p < .01$), and a significant correlation between the decrease in GMV and WMV was not observed ($r = -0.071$, $p < .53$). However, the difference between the pairs of correlated correlations was not found to be significant.

INSERT FIGURE 3 APPROXIMATELY HERE

In light of the significant impact that TOD has on the apparent volume of the three main tissue compartments, it is important to test whether specific gray matter, white matter, and ventricular regions were more susceptible to the TOD effect (Table 1), as this analysis could provide some clues about its likely

biological origins. With regard to gray matter regions, the TOD effect was most obvious in cortical gray matter volume (−1.49% of ICV per 24 h), and, to a lesser extent, in the cerebellar cortex (−0.166% of ICV per 24 h). Interestingly, with the exception of the hippocampus (−0.009% of ICV per 24 h), none of the subcortical structures showed a significant TOD effect. With regard to the specificity of the TOD effect within white matter regions (Table 1), a significant effect of TOD was found to be restricted to the middle posterior region of the corpus callosum (−0.002% of ICV per 24 h) and to the optic chiasm (−0.003% of ICV per 24 h), as well as to cortical white matter (−0.487% of ICV per 24 h). In contrast, all of the subcompartments that contribute to total CSF volume showed a significant effect of TOD at $q < .05$.

With regard to CSF (Table 1), the most impacted region was observed in the lateral ventricles (0.062% of ICV per 24 h), followed by the third ventricle (0.009% of ICV per 24 h). A comparable impact on measured volume due to TOD was found in the fourth ventricle (0.013% of ICV per 24 h) as well as in the CSF compartments associated with cortical sulci (0.008% of ICV per 24 h) and in the inferior lateral ventricle (0.005% of ICV per 24 h). Tissue classified as choroid plexus (0.006% of ICV per 24 h) was the least impacted of the CSF volumetric regions. Finally, the effect of TOD was also observed in terms of a decrease in the volume of the brain stem (−0.036% of ICV per 24 h).

INSERT FIGURE 4 APPROXIMATELY HERE

Considering the striking decrease in volume observed in the cortical gray matter (i.e., gray matter ribbon of the cerebral cortex), we next tested whether specific surface-based morphometric measures generated by FreeSurfer are more prone to be confounded by the TOD effect. As shown in Table 2, with TOD as a fixed effect, the LME analysis revealed a significant reduction in apparent cortical thickness (−0.092 mm per 24 h) and apparent surface area (−693.5 mm² per 24 h) measurements of the cerebral cortex. None of the other surface-based measures tested showed a similar effect of TOD. In light of the marked effect of TOD on apparent cortical thickness, we probed whether there are specific brain

regions within the cortical ribbon where TOD has a greater impact on the measurement of apparent cortical thickness. As shown in Figure 4a, the ROI analysis based on the Desikan-Killiany parcellation scheme revealed a dorsal-ventral gradient in the rate of change in apparent cortical thickness: regions along the frontal and temporal lobes showed a greater decrease in cortical thickness compared with the occipital and parietal lobes, which showed a trend towards an increase in cortical thickness (see Supplementary Figure 3). Indeed, the most significant TOD-related changes in cortical thickness (Figure 4b) were observed along the midline and dorsal regions of the frontal lobe (superior and middle frontal gyri as well as the paracingulate gyrus), followed by changes in the dorsal-lateral portions of the temporal lobe (superior and middle temporal gyri). There was no evidence of hemispheric lateralization of these TOD effects. We therefore tested whether the TOD effect is specific to particular lobes in the brain. The ANOVA revealed a significant interaction between TOD and Lobe (Cingulate, Frontal, Insula, Occipital, Parietal, and Temporal) ($F = 2.94$; $p < .0167$) (Figure 5). Subsequent post hoc tests (paired T -test) revealed that the TOD effect on the frontal lobe was significantly greater compared with all the other lobes, with the exception of the temporal and cingulate lobes. Similarly, the impact of TOD on the temporal lobe was significantly greater compared with the impact on the parietal and insula regions, but not with that of the frontal, cingulate, and occipital regions. These results provide further evidence of a disproportionate effect of TOD on specific brain lobes.

INSERT FIGURE 5 APPROXIMATELY HERE

VBM Analysis: The whole-brain VBM analysis based on processing the T₁W images through the FSL-longitudinal pipeline revealed significant clusters of reduction in gray matter density due to TOD along the midline regions in the frontal cortex, as well as in the bilateral middle frontal lobe, the superior parietal lobe, and the left superior temporal lobe (Figure 6). In addition, the VBM analysis revealed significant reductions in gray matter density in subcortical structures including the cerebellum and the caudate nuclei. These results are in agreement with the findings from the cortical thickness analysis, although some differences

can also be observed. Such inconsistencies are not surprising given that the VBM analysis is based on a voxelwise assessment of volume differences while the FreeSurfer based measures of volume were based on the average volume of an entire brain structure delineated by the automatic parcellation procedure.

INSERT FIGURE 6 APPROXIMATELY HERE

DISCUSSION

The findings from the present study provide clear evidence for a significant impact of TOD on measures of brain morphometry derived from T₁W images. Our results show that (a) TOD impacts measures of brain morphometry computed from T₁W images even in a relatively small group of healthy adult volunteers; (b) the apparent volume of all three major tissue compartments—GM, WM, and CSF—are influenced by TOD; however, the magnitude of the TOD effect varies across these tissue compartments, with the most striking difference in volume observed in CSF, followed by significant differences in GMV and WMV respectively; (c) the increase in CSF volume is associated with the decrease in GMV and WMV; (d) measures of cortical thickness, cortical surface area, and gray matter density were all affected by TOD, while other measures such as curvature indices and sulcal depth, were not impacted; (e) the effect of TOD appears to have a disproportionate impact on morphometric measures of the frontal and temporal lobe compared with other major lobes of the brain; (f) and finally, the impact of TOD on brain morphometry cannot be explained by factors such as subject motion or a change in signal to noise ratio (SNR) from AM to PM (see Supplementary Materials).

That gross measures of brain volume change because of TOD is in agreement with the main finding from Nakamura et al. (2015). In that study, the authors reported that from morning to evening TBV, as measured using T₁W images, differed by −0.09% of TBV in a large group of patients with multiple sclerosis and −0.221% in a large group of individuals with and without dementia. In the present study, we found that the decrease in TBV from morning to evening can be detected even in a relatively small group of healthy young adults with high

statistical significance. However, unlike the rate of decrease measured in the clinical and elderly groups by Nakamura and colleagues, we estimate an even greater difference in the brain volume of young healthy adults of -1.145% of *ICV per 12 h*, roughly five times the magnitude of the effects reported so far. This finding suggests that the TOD effect can significantly confound the measurement of brain volume even with a sample size that is typical of clinical research studies.

The reduction in apparent brain volume due to TOD can be observed in 16 out of the 19 participants, but there is also considerable individual variability (Supplementary Figure 1). This is consistent with the findings from Maclaren et al (2014) who found a significant positive correlation between TOD and the change in volume of the lateral ventricles in one subject, while in the remaining two subjects, the correlation was not statistically significant. In addition, the effect of TOD on subcortical structures like the hippocampus is consistent with recent reports that seasonal differences in the length of a day (i.e. photoperiod) have an impact on hippocampal volume (Miller et al., 2015). Taken together, the results from our study and those from the existing literature suggest that the decrease in brain volume is generalizable across different control and patient groups and can be detected despite differences in technical factors including image acquisition protocol, scanner type, scanner field strength, SNR, and head motion, providing support to the notion that the TOD effect has a physiological origin.

Although our study was not designed specifically to draw inferences about the underlying biological mechanisms of the TOD effect, it is worthwhile to consider some of the likely candidates. The strong correlation between changes in CSF volume and changes in GMV and WMV from morning to evening suggests that the TOD effect may be primarily driven by the regulation of CSF in the brain. CSF is predominantly generated by the choroid epithelial cells found in all four ventricles of the brain, although bulk flow of interstitial fluid from brain parenchyma to the ventricles and subarachnoid space is known to account for approximately 10 to 30% of the total CSF production (Cserr, 1984). The physiological mechanisms regulating the secretion and flow of CSF remain to be

fully characterized, but there is growing recognition that CSF dynamics in the brain are mediated by a cascade of events involving a wide array of hormones and neuropeptides and their interaction with the vascular system (for a review, see Chodobski and Szmydynger - Chodobska, 2001). With respect to the TOD effect, a survey of the literature on factors that regulate CSF volume raises several possible factors such as levels of hydration and circadian rhythm.

Hydration and the TOD Effect

One possibility is that dehydration from morning to evening may account for the TOD effect. However, it is worth noting that our experiment design required subjects to take a lunch break before the afternoon scan, which would typically reduce the likelihood of subjects being dehydrated. But there are some additional important differences worth considering between our findings and those from studies that have explored the relationship between dehydration and brain volume. Studies on the effects of hydration on the brain have found that acute dehydration evoked by strenuous exercise does not have an impact on total brain volume but resulted in a subtle, yet significant increase in ventricular volume (Dickson et al., 2005; Kempton et al., 2009). However, Duning et al. (2005) found that in young healthy adults dehydration induced by avoiding fluid intake for 16 h was found to decrease brain volume by 0.55% while acute rehydration increased brain volume by 0.72%. Overall, while hydration appears to have a significant impact on ventricle volume, the concomitant impact on total brain volume is at levels that either are not detectable or require extreme manipulation of hydration levels. In this respect, the magnitude of the changes in total brain volume observed in the present study are not in agreement with changes evoked by hydration status. More recently, using the automated methods that we used in the present study Streiburger et al. (2012) explored the effect of hydration on the volume of the three major tissue compartments in the brain. Consistent with previous studies, Streiburger et al. found that dehydration resulted in an increase in the entire ventricular system. Interestingly, dehydration was found to be associated with a significant decrease in cortical white matter volume, but neither

acute dehydration nor hyperhydration evoked any significant changes in cortical GMV or apparent cortical thickness as measured with FreeSurfer. The only effect of dehydration on GMV was restricted to a GMV decrease in subcortical regions in close proximity to the ventricles. In contrast, our results suggest that TOD has a significant impact on measurement of GMV, which manifests as a significant decrease in apparent cortical thickness. Secondly, our results indicate that the magnitude of the TOD effect was relatively smaller in WM compared with GM and CSF. These differences in the results between the hydration studies and the present study suggest that changes in hydration from morning to evening may not fully account for the TOD effect.

Evidence from multimodal MRI studies of the TOD effect

The few MRI studies that have examined diurnal changes reveal some consistency in the topography of the brain regions that appear to be impacted by TOD. For example, the results from the DTI study (Jiang et al., 2014) indicate an apparent increase in FA that was focused along the midline regions in the frontal cortex (i.e., the superior frontal gyri, the paracingulate gyri) and regions close to sulci. However, the increase in DTI metrics such as ADC, RD, and AD were focused predominantly along the occipital and temporal cortices, regions where we observed an increase in cortical thickness from morning to evening (See Supplementary Figure 4). Interestingly, using PET, Buysse et al. (2004) found that subjects showed increased glucose metabolism in the occipital and temporal lobes in the morning compared with the evening. The opposite contrast (i.e., evening > morning) showed increased glucose metabolism in several midbrain regions such as the tegmentum, precentral gyrus, superior temporal gyrus, and posterior hypothalamus.

Another possibility is that changes in rCBF from morning to evening could account for the TOD effect. Hodkinson et al. (2014) tested this possibility in a group of young, healthy male volunteers using whole-brain rs-fMRI and ASL. They found that the TOD-related decrease in functional connectivity and rCBF overlapped in several brain regions along the midline, such as the anterior

cingulate cortex, the posterior cingulate cortex, and the inferior parietal lobule. Furthermore, the decrease in rCBF correlated with an increase in salivary cortisol levels. Given that cortisol levels are regulated by the circadian rhythm (Van Cauter et al., 1996), these findings provide further support to the link between circadian rhythm and hemodynamic changes in the brain. A common thread that links our findings with the existing literature is that across studies that employed different MRI methods, dorsal regions of the frontal lobe particularly along the midline appear to be affected by TOD (Supplementary Figure 4). That the changes in apparent cortical thickness and gray matter volume that we detected appear to be related to changes in CSF demands further consideration of the relation between circadian rhythm, CSF regulation, and cerebral blood flow.

Relation between circadian rhythm, CSF regulation, and the TOD effect

Observations from studies involving patients with hydrocephalus have shown that ventricular fluid pressure fluctuates on an hourly and daily basis and is associated with physiological factors (e.g., cardiac pulsation and respiration) as well as general body activities (Hayden et al., 1970). Consistent with these reports, MRI-based phase imaging studies of the cerebral aqueduct in healthy volunteers have shown that CSF production varies widely during the day, but the lowest production was observed between 4PM and 6PM (12 ± 7 ml/h), which ramped up in the evening and peaked during the night between 11PM and 2AM (42 ± 2 ml/h) (Nilsson et al., 1994; Nilsson et al., 1992). Although CSF production during the morning hours was not recorded in this study, on the basis of these observations one would expect to measure an increase in total CSF volume in the morning that declines over the day, which is contrary to our findings (Figure 2d).

The dynamics of CSF volume in the brain involves not only the production, but also the reabsorption of CSF during the course of a day (Cutler et al., 1968). CSF is mostly reabsorbed via arachnoid granulations that protrude through the dura mater and are found predominantly along the walls of the venous sinuses (Supplementary Figure 5) (Grzybowski et al., 2007) with the superior sagittal

sinus as the principle drainage route (Upton and Weller, 1985). Interestingly, differences in hydrostatic pressure between CSF and sagittal sinus blood flow have been found to drive the absorption of the CSF through the arachnoid granulations (Bering and Salibi, 1959). The results from our cortical thickness and VBM analyses consistently show the effects of TOD primarily in the superior frontal and the paracingulate gyri (see Figures 4b and 5b), regions that abut the superior sagittal sinus and the inferior sagittal sinus, respectively. In addition, the clusters showing significant reduction of gray matter density tend to border the major gyri, such as the pre-and postcentral gyri, the lateral sulcus, and the superior temporal gyrus. Thus, one plausible interpretation is that the effect of TOD that we observe may be related to the diurnal absorption of CSF via the arachnoid granulations distributed along the walls of the venous system.

The TOD effect as a biological phenomenon

In this respect, the phenomenon we observe here may be related to the “glymphatic system” described as the pathway by which toxic metabolic waste that accumulates in brain tissue is removed by the convective exchange of CSF with the interstitial fluid (Nedergaard, 2013). The activation of the glymphatic system, however, is determined by the sleep-wake cycle (Xie et al., 2013). Although the arousal status of the participants in our study was not continuously monitored, it is unlikely that participants were asleep during the T₁W scans since these images were acquired in the beginning of the scan sessions, and participants were reminded to stay still before each scan. That said, a noteworthy finding that emerged from studies that investigated the glymphatic system in mice was that even though CSF influx into the brain is greatly reduced during wakefulness, it does permeate the superficial cortical layers (Xie et al., 2013). Moreover, lymphatic vessels that carry out the function of draining interstitial fluids to the deep cervical lymph nodes have been described to be highly concentrated along the walls of the dural sinuses (Louveau et al., 2015). Whether the magnitude of this CSF influx during wakefulness is regulated by the circadian rhythm is an open question. Nevertheless, in a T₁W image at a voxel resolution of 1 mm³ this influx of CSF may manifest as a partial volume effect that, in turn,

impacts measurement of gray matter volume, given the proximity of the cortical ribbon to the subarachnoid space. This phenomenon may also explain why measurement of GMV is more affected than WMV.

Even though currently we can only speculate about the exact biological mechanism, such diurnal fluctuations in brain volume appear to be a crucial part of brain homeostasis, and perturbations in the rate of change of brain volume measures during the course of a day can be indicative of brain disorders. Indeed, our findings suggest that in healthy young adults, the TOD-related change in brain volume was -1.14% of ICV (per 12 h), whereas in patients with multiple sclerosis as well as in elderly individuals with and without dementia (-0.090% and -0.221% per 12 h, respectively) the change in brain volume was greatly attenuated (Nakamura et al., 2015). In light of the growing recognition of the role that CSF flow plays in removing toxic metabolic waste products from the brain (Iliff et al., 2014), further investigations elucidating the underlying biophysical mechanisms of the phenomenon we report here are clearly warranted.

Implications of the TOD effect

In the context of studies that use T₁W morphometry to understand the relation between changes in brain structure and behavior, it is also clear that the TOD effect is a confound that cannot be ignored. Thus, in cross-sectional studies, if experimental constraints biased the acquisition of MRI data from the control group towards the morning and the clinical group towards the evening, the TOD effect could be misinterpreted as a pathological reduction in brain volume (Nakamura et al., 2015). Likewise, in longitudinal designs, which are typically used to test the effects of behavioral training on brain structure, biases in the time of acquisition of the MRI data could result in significant differences in apparent brain volume that could be misattributed to the effects of training. For example, if there was a tendency for the baseline scan to be acquired in the afternoon and the post-training scan in the morning, an increase in apparent brain volume would be detected, while the reverse could manifest as a decrease in apparent brain volume. Indeed, both increases and decreases in brain volume

have been reported in the literature as an effect of brain training (Boyke et al., 2008; Draganski et al., 2004; Taubert et al., 2010).

There are several important factors to consider with regard to the implication of the TOD effect for studies of structural plasticity. First, the effect size of plasticity studies appears to be smaller than the average effect of TOD observed in our study. For example, 8 weeks of memory training were found to increase cortical thickness by ~0.05 mm in the right insula (Engvig et al., 2010), whereas the average change in cortical thickness due to the TOD effect is -0.092 mm. Second, our results suggest that among the different measures of brain morphometry derived from T₁W images, measures of apparent cortical thickness, cortical surface area, and gray matter density—all widely used structural metrics of the brain—are particularly impacted by TOD. That such significant differences can be observed despite using the automated FreeSurfer longitudinal stream, which is known to reduce within-subject variability (Reuter et al., 2012), bring into sharp focus the limitations of T₁W morphometry in providing accurate measures of brain morphometry. Finally, our results reveal a specific spatial topography to the TOD effect on these morphometric measures, with the cortical regions along the frontal and temporal lobes showing a disproportionately greater change due to TOD, while regions along the parietal and occipital lobes show an opposite pattern (see Supplementary Figure 3). As a result, if the TOD effect is not accounted for in the statistical analyses, the resulting differences in brain morphometrics can be misinterpreted as the effect of certain experimental manipulations. Indeed, controlling for the TOD effect by adding it as a covariate in the statistical model has been suggested as one way to improve statistical inference (Nakamura et al., 2015).

Conclusion

Taken together, the numerous measures of brain structure that can be computed from T₁W images, although of great importance for advancing our understanding of the relation between brain structure and function, are influenced by several factors that affect the MRI signal. Our findings reveal that the TOD effect impacts

automatically derived measures of brain morphometry computed from T₁W images that are thought to reflect structural metrics, such as cortical thickness, surface area, gray matter volume, and gray matter density. While this effect may be considered a confound in longitudinal or cross-sectional experiments that should be controlled for, our results also suggest that the TOD effect is a physiological phenomenon that merits further investigation.

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TABLES

Table 1

Volumetric Region	Intercept	(SE)	TOD Slope	TOD (SE)	q
Gray Matter					
Accumbens Area	0.036	0.001	-0.002	0.001	0.125
Amygdala	0.093	0.002	0.000	0.002	0.940
Caudate	0.269	0.008	-0.004	0.002	0.121
Cerebellum Cortex	3.596	0.078	-0.166	0.034	0.000
Cortical Gray Matter	33.220	0.450	-1.490	0.283	0.000
Hippocampus	0.268	0.007	-0.009	0.002	0.010
Pallidum	0.119	0.003	0.000	0.002	0.975
Putamen	0.376	0.011	0.003	0.004	0.603
Thalamus	0.567	0.011	-0.005	0.004	0.325
Ventral Diencephalon	0.268	0.006	-0.005	0.003	0.161
White Matter					
Cerebellum White Matter	1.016	0.032	0.010	0.024	0.765
Corpus Callosum - Anterior	0.056	0.002	0.000	0.001	0.975
Corpus Callosum - Central	0.028	0.001	-0.002	0.001	0.107
Corpus Callosum - Middle Anterior	0.029	0.001	-0.002	0.001	0.125
Corpus Callosum - Middle Posterior	0.026	0.001	-0.002	0.001	0.019
Corpus Callosum - Posterior	0.057	0.001	-0.002	0.001	0.109
Cortical White Matter	28.969	0.504	-0.487	0.142	0.010
Optic Chiasm	0.018	0.001	-0.002	0.001	0.018
CSF					
3rd Ventricle	0.053	0.003	0.009	0.002	0.000
4th Ventricle	0.134	0.008	0.013	0.003	0.000
Choroid Plexus	0.097	0.004	0.006	0.003	0.041
CSF (sulcal)	0.060	0.003	0.008	0.002	0.000
Inferior Lateral Ventricle	0.021	0.002	0.005	0.001	0.001
Lateral Ventricles	0.398	0.038	0.062	0.009	0.000
Miscellaneous					
Brain Stem	1.523	0.034	-0.035	0.010	0.010
Vessel	0.006	0.001	0.001	0.001	0.109

Intercept values are in % of ICV

Slope changes are in % of ICV per 24 h

q-values of TOD effect are based on the false-discovery rate adjustment of *p* values

The intercept and slope values showing the effect of TOD on gray matter, white matter, and ventricular regions

Table 2

Surface Measurements	Intercept	(SE)	TOD Slope	TOD (SE)	q
Cortical Thickness (mm)	2.565	0.022	-0.092	0.020	0.001
Curvature					
Fold Index*	3910.300	991.200	486.300	1824.800	0.870
Curvature Index*	394.540	31.580	-16.070	54.850	0.870
Gaussian Curvature (mm ²)	0.059	0.018	0.042	0.034	0.582
Mean Curvature (mm)	0.141	0.001	0.001	0.002	0.837
Gyrus Height (mm)	0.438	0.003	-0.002	0.003	0.837
Sulcal Depth (mm)	0.519	0.003	0.000	0.003	0.870
Surface Area (mm²)	88977.700	1989.500	-693.500	274.300	0.049

*Indicates unitless measurement

Slope changes are in a 1 measurement-unit per 24 h

q-values of TOD effect are based on the false-discovery rate adjustment of *p* values

The intercept and slope values showing the effect of TOD on the different types of morphometric measures derives from processing T₁W images with FreeSurfer.

FIGURES

Fig 1. *An illustration of the experiment design adopted for the present study. Each participant was scanned across four consecutive visits. During each visit the participants were scanned in the morning and in the afternoon. Between the two scan sessions, participants were asked to rest during Visits 1 and 4 but were given a visuo-motor training task during Visits 2 and 3. Participants were not given any specific training between Visits 1 and 3, but after Visit 3 they were given ~45 minutes of training every day for four to five consecutive days on the visuo-motor task that was introduced to them on Visit 3. Given that Visits 1 and 4 did not include a training intervention between scan sessions, we used the data from these visits to explicate the impact of TOD on brain morphometrics. In comparison, the data from Visits 2 and 3 were used primarily to demonstrate that our findings can be reproduced even in the context of a typical study examining acute changes in brain structure following behavioral training.*

Fig. 2. *Model-corrected (Eq. 1) changes in total brain volume, total gray matter volume, total white matter volume, and total CSF volume expressed as percentages of ICV and plotted against the TOD from the primary dataset (152 scans from 19 subjects). Volumetric measures were adjusted for the random effect of subject intercept. The green lines indicate the model estimate of the fixed effect of TOD and the light gray region surrounding the green line shows the 95% confidence interval of the fitted model. The blue circles indicate individual measurements. There was a significant effect of TOD on (a) total brain volume (-1.145% of ICV per 12 h, $p < 1.0 \times 10^{-10}$); (b) total gray matter volume (-0.929% of ICV per 12 h, $p < 1.0 \times 10^{-8}$); (c) total white matter volume (-0.244% of ICV per 12 h, $p < .0007$); (d) total CSF volume ($.085\%$ of ICV per 12 h, $p < 1.0 \times 10^{-11}$).*

Fig. 3. *Pearson's correlations between changes in the three main tissue compartments with TOD. CSF volume change significantly correlated with GM volume change ($r = -0.527$, $p < 1.011 \times 10^{-6}$), as well as WM volume change ($r = -$*

0.293, $p < .01$). A significant correlation between GM volume change and WM volume change was not observed ($r = -0.071$, $p < .53$).

Fig. 4. Apparent decrease of cortical thickness as a function of TOD plotted across 68 parcellated cortical ROIs (34 per hemisphere) by using the primary dataset. (a) The rate of the change in cortical thickness due to the TOD effect. Light blue/dark blue indicates the thickness loss per day. Dark blue regions correspond approximately to a thickness loss of 0.1 mm per 12 h. (b) Cortical regions that show a significant decrease in apparent cortical thickness from morning to afternoon after correcting for multiple comparisons (FDR $q < .01$). Abbreviations: mFG: Middle Frontal Gyrus; sFG: Superior Frontal Gyrus; iFG: Inferior Frontal Gyrus; mTG: Middle Temporal Gyrus; sTG: Superior Temporal Gyrus; pCingG: Paracingulate Gyrus; CG: Cingulate Gyrus; AnG: Angular Gyrus.

Fig. 5. The average difference in cortical thickness from morning and afternoon scanning sessions in the six major cortical regions. TOD biases the measurement of apparent cortical thickness specifically in regions like the frontal and temporal lobes. (* $p < .05$)

Fig. 6. Clusters show significant gray matter density reduction with TOD. Clusters shown survived a voxelwise uncorrected threshold level of $p < .0001$, followed by a cluster correction at a threshold of $p < .0001$ to account for multiple comparisons. Abbreviations: cOperC: Central Opercular Cortex; Crblm: Cerebellum; pCingG: Paracingulate Gyrus; PreCun: Precuneus; PreCG: Precentral Gyrus; CdN: Caudate Nucleus; postCG: Post Central Gyrus; mFG: Middle Frontal Gyrus; sTS: Superior Temporal Sulcus; sFG: Superior Frontal Gyrus; sMarG: Supramarginal Gyrus; LV Lateral Ventricle; sPL: Superior Parietal Lobe.

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