SHORT NOTE

White Matter Differences Predict Cognitive Vulnerability to Sleep Deprivation

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Objectives: In other disciplines, white matter (WM) differences have been linked to cognitive impairments. This study sets out to clarify whether similar microstructural differences in WM tracts predict a person's cognitive vulnerability to the effects of total sleep deprivation (TSD).

Design: Participants completed a simple visual-motor task both before and after 24 h of TSD. Using a median split on the percent change in accuracy from pre-TSD to post-TSD, participants were separated into susceptibility groups. A diffusion tensor MR imaging (DTI) scan was acquired from each participant, and fractional anisotropy (FA) was calculated, examined across the brain, and compared between susceptibility groups.

Setting: University of Texas at Austin.

Participants: Thirty-two West Point cadets (9 females, 23 males) between 19 and 25 years of age.

Results: Participant susceptibility to TSD was correlated with lower FA values in multiple regions of white matter, including the genu of corpus callosum and ascending and longitudinal white matter pathways. Significantly higher FA values in those less vulnerable to TSD, indicating increased neural connectivity and WM organization, may moderate the cognitive effects of sleep deprivation.

Conclusions: Differences in distributed WM pathways reflect, and may contribute to, a person's ability to function effectively when sleep deprived. The widespread nature of this effect supports previous views that TSD has a global effect on brain functioning.

Keywords: Sleep deprivation; magnetic resonance imaging; cerebral cortex; cognition

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RESEARCH IN THE PAST 5 YEARS HAS REFLECTED INCREASING INTEREST IN UNDERSTANDING INDIVIDUAL DIFFERENCES THAT CONTRIBUTE TO VULNERABILITY to sleep deprivation. One study used regression models that included individual differences and accounted for nearly 83% of the variance in cognitive impairment due to sleep deprivation over a 14-day period.[1] In contrast, models that did not include individual differences accounted for only 22% of the variance.

To further investigate individual differences, researchers have looked to the neural correlates of sleep deprivation vulnerability. Utilizing functional magnetic resonance imaging (fMRI) with a verbal working memory task, researchers found an overall decrease in both regional and global neural activation after 30 h of sleep deprivation.[2] In addition, decreases in neural activity were uniquely correlated with participants' performance, such that as numbers of errors and reaction times (RTs) increased, brain activation tended to decrease. This finding indicates that it is possible to uncover neural correlates of individual differences in response to sleep deprivation.

To date, examinations of the neural correlates of sleep deprivation have been restricted to functional techniques (e.g., fMRI and electroencephalography [EEG]). However, other research domains have begun to reveal significant structural brain correlates of individual differences in cognitive abilities. One of the domains that has been studied extensively, in which changes in cognitive ability have been linked to brain structure, is aging. A recent examination of both cortical gray and white matter (WM) changes associated with aging demonstrated a significant main effect of age across both tissue types, but it was only a measure of WM that revealed significant correlations with cognitive performance.[3] The WM measure utilized in this study was obtained with diffusion tensor MR imaging (DTI), in which a scalar measure of diffusion, referred to as fractional anisotropy (FA), was calculated and tested. FA is thought to reflect in part, the number of fibers, myelination, and compactness of WM tracts.[4] In the aging study, reduced FA values in functionally specific regions were found to reflect lower performance on tasks of cognitive control and episodic memory. Other studies have demonstrated that even in healthy young persons, differences in the microstructure of WM tracts can be correlated with cognitive processing abilities.[5] Therefore, it is possible that the ability to function cognitively under conditions of sleep deprivation could be related to differences in WM microstructure.

The current study examined whether differences in WM structure may help explain important individual differences in vulnerability to sleep deprivation. Using DTI, we indexed WM FA values of 32 West Point cadets who underwent 24 h of total sleep deprivation (TSD). A simple visual-motor control (VMC) task was used to separate participants into vulnerability groups.

METHODS

Participants

Thirty-two West Point cadets (9 female) between the ages of 19 and 25 (M = 20.8 years) participated as part of a larger study on the effects of TSD on cognition that included fMRI.[6] Participants were screened to be free from prior history of neurological or psychiatric illness or current psychotropic or sleep related medication. Cadets who were unable to stay awake and perform at 75% accuracy or better on the VMC task after 24 h of TSD were excluded. Five cadets were excluded based on

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these criteria and another 3 were removed from the final analysis due to poor image quality. The final analyzed group consisted of 24 West Point cadets (7 female) between the ages of 19 and 25 (M = 20.8 years). The Institutional Review Board at the University of Texas at Austin and West Point Military Academy approved the study, and informed consent was obtained from all participants.

Behavioral

Groups of 3 West Point cadets participated in each study session. Participants were not allowed to consume alcohol 24 h prior to the study or to consume caffeine between midnight and 06:00 before the first or second day. They were instructed to engage in normal sleep-wake cycles prior to arriving in Austin for the study and the night before the actual run. Compliance on this was peer monitored. Participants arrived for MRI scanning and other cognitive tasks at 06:00 on the morning of Day 1. DTI and high-resolution T1 scans used in this analysis were performed at this session. After a full day of testing, which included afternoon physical endurance testing, an assigned monitor accompanied the cadets at all times. During the evening and night, they ate meals and engaged in both light physical and mental activities such as walking, bowling, and video/board games to keep them awake. After breakfast on Day 2, they returned for more MRI and cognitive tasks at 06:00.

On each morning participants engaged in an fMRI protocol designed to assess neural changes associated with sleep deprivation on decision-making. The fMRI component had 4 conditions: a visual-motor task (VMC) that was used as a control task and 3 decision-making tasks. The different tasks were organized in a block design and were presented in counterbalanced orders, with the VMC task separating each decision-making task throughout a 26-min scan session. In the VMC task, participants were asked to indicate with a response button on which side of the screen an 'X' was presented. A total of 160 VMC trials, split into 4 runs, were completed in each experimental session, one before and one after 24 h of TSD. Performance declines on the 3 decision-making tasks did not reveal reliable WM differences using whole brain analysis. However, performance changes on the VMC task did reveal widespread significant differences, and therefore are the focus of this report (see online supplement for more detail on the additional decision making tasks and procedures).

Imaging

Two high-resolution SPGR, T1-weighted scans were collected using a 3 Tesla GE MRI equipped with an 8-channel phased array head coil (GE Medical Systems, Milwaukee, WI) with the following image parameters: TR/TE = 5.0/1.2, flip angle = 11°, slice thickness = 1.3 mm, 172 slices, FOV = 256 × 256 mm. The two T1 scans were first motion corrected and then averaged to create a single high-signal, high-contrast volume.

Whole brain DTI was also acquired for 25 directions using a dual shot echo planar imaging and a twice-refocused spin echo pulse sequence, optimized to minimize eddy current-induced distortions (GE 3T, TR/TE = 12000/71.1, B = 1000, 128 × 128 matrix, 3-mm (0 mm gap) slice thickness, 0.94 × 0.94 mm in plane resolution, 1 T2 + 25 DWI). Forty-one slices were acquired and diffusion tensors and FA values were calculated on a voxel-by-voxel basis using conventional reconstruction methods in FSL.[7] Individual FA images were registered in MNI-152 standard space using FMRIB's Nonlinear Registration Tool (FNIRT), a part of the Tract Based Spatial Statistics (TBSS) software.[8] This approach is based on calculating FA skeletons that ensure that each participant's WM tracts were accurately represented in both group and individual space. Whole brain group differences in WM tracts were analyzed using a permutation method with the α level set at $P < 0.01$ (corrected using threshold-free cluster enhancement (TFCE);[9] see supplement for more details on the imaging analysis methods).

RESULTS

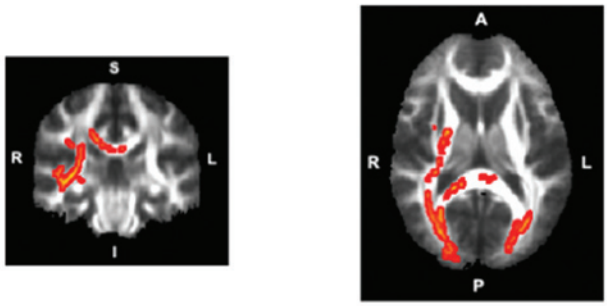
Behavioral

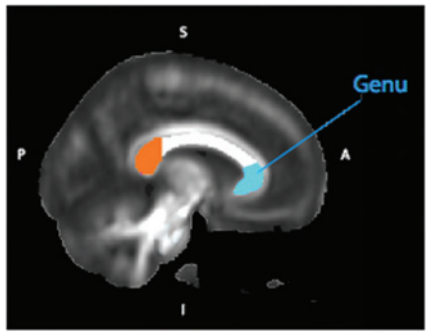
To index decline in cognitive performance caused by sleep deprivation, a percent change score was calculated for the VMC task ((Day 2 VMC accuracy - Day 1 VMC accuracy) / Day 1 VMC accuracy). Participants were divided, using a median split, into those who were more vulnerable (VUL, n = 12, M = −6.5%) and those who were less vulnerable (NON, n = 12, M = −1.5%) to TSD. There were no differences in age, $\chi^2(5, N = 24) = 8.70$, $P > 0.05$, or gender distribution, $\chi^2(1, N = 24) = 0.202$, $P > 0.05$, across either of the 2 groups. In addition, there were no differences between groups in baseline performance on Day 1, (mean proportion correct VUL = 1.0 (SE = 0.002), or mean NON = 1.0 (SE = 0.001), $F_{1,23} < 1$).

WM Differences

A whole brain comparison of FA values that contrasted NON > VUL indicated multiple WM pathways where FA values were significantly greater for the NON group relative to the VUL group. There were no significant pathways where VUL demonstrated greater FA values than NON (Figure 1a). A percent change score was also calculated separately for each of the 3 decision-making tasks used in the fMRI study (excluding missed trials) using the same method as with the VMC task. When vulnerability groups were defined, based on performance declines on these "higher order" decision tasks, none of these whole brain comparisons of FA values showed any significant differences.

Visual examination of the VMC vulnerability analysis revealed greater effects in the right hemisphere (Figure 1a). To assess the reliability of this apparent finding, as well as to probe the results for a linear relationship between FA and VMC change, mean FA values were extracted from each individual's FA skeleton map. This was accomplished by using masks defined by the Johns Hopkins University (JHU) DTI-based white matter atlases available in FSL (see supplement for more procedural detail and Figure 2 in the supplement for the corresponding WM pathways). FA values were tested in a 2 × 2 × 11 repeated measures ANOVA with hemisphere and region as within-subjects factors and group as a between-subjects factor. The results revealed a significant main effect of group ($F_{1,22} = 16.93$, $p < 0.001$), hemisphere ($F_{1,22} = 67.70$, $p < 0.001$), region ($F_{10,22} = 628.10$, $p < 0.001$), and a hemisphere by region interac-

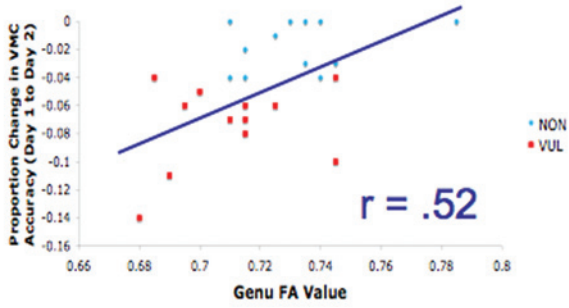




Vulnerability Group

Region	VUL FA	NON FA	Pearson correlation	
Posterior limb of internal capsule	0.68	0.70	0.56	
Retrolenticular part of internal capsule	0.61	0.64	0.45	
Superior corona radiata	0.48	0.50	0.34	
Posterior corona radiata	0.48	0.51	0.49	
Forceps major	0.66	0.70	0.6	
Corticospinal tract	0.62	0.63	0.39	
Posterior thalamic radiation	0.64	0.66	0.49	
Superior longitudinal fasciculus	0.51	0.53	0.45	
Genu of CC	0.71	0.74	0.52	
Body of CC	0.62	0.65	0.26	
Splenium of CC	0.80	0.83	0.39	
Mean FA	0.62	0.64		p < .05

A



B

Figure 1—A) Across the top row, from left to right, is one coronal and one axial brain slice from the whole brain Tract Based Spatial Statistics (TBSS) results, highlighting those regions that showed those less vulnerable to sleep deprivation (NON) greater than those more vulnerable to sleep deprivation (VUL) in fractional anisotropy (FA) differences ($P < 0.01$, corrected). For purposes of visualization, significant portions of the tested white matter skeleton were expanded to fill more of the corresponding white matter tract. Table of mean FA values for the extracted pathways of interest and their correlations with percent change in visual-motor control (VMC) accuracy between Day 1 and Day 2. **B)** From top to bottom is one sagittal slice showing the 3 regions of the corpus callosum (CC) that were analyzed for FA differences. Graph showing the correlation between the FA values extracted from the genu of the CC and the proportion change in VMC accuracy between Day 1 and Day 2.

tion ($F_{10, 22} = 6.10$, $p < 0.001$). This latter interaction revealed that some posterior regions showed a right > left laterality in FA values, something that has been found in past studies.[10] Most critically, there was no hemisphere by group interaction and no region by group interaction, thereby indicating that the visually apparent laterality was not a reliable effect. To test whether total WM volume, age, or sex could be contributing factors to these results, each variable was entered separately as a covariate in the ANOVA analysis. Using total WM volume as a covariate, the effect of group remained highly significant ($F_{1, 21} = 15.12$, $P < 0.001$), with no main effect of WM volume ($F < 1$). The same basic finding was true for both age (main effect of group, $F_{1, 21} = 30.10$, $P < 0.001$) and sex (main effect of group, $F_{1, 21} = 15.87$, $P < 0.001$). Of the age and sex variables, only age showed an additional main effect ($F_{1, 21} = 9.77$, $P < 0.01$), reflecting increasing FA values with increasing age.

FA values from those regions identified as significant by the TBSS analysis were tested for a linear relationship with the VMC percent change score. Nearly all regions showed a significant linear relationship (see Figure 1a), but given the ceiling effect for performance of the NON group, this relationship is likely driven primarily by the VUL group (see Figure 1b for the correlation results for the genu of the corpus callosum (CC)).

DISCUSSION

The current study evaluated whether differences in WM are associated with cognitive vulnerability to TSD over a 24-h period. FA, an index of WM organization, was significantly correlated with an individual's vulnerability to TSD as measured by change in performance on a simple visual-motor task. Specifically, higher FA values in the posterior limb and retrolenticular part of the internal capsule, posterior corona radiata, forceps major, posterior thalamic radiation, superior longitudinal fasciculus, and the genu of the corpus callosum all seem to be associated with decreased vulnerability to TSD. These results were not reflected in changes associated with TSD across any of the "higher order" decision-making tasks, in which no relationship between performance and FA were found. In general, FA values have been found to be higher in regions with restricted diffusion—regions with highly organized structure—and lower in regions with less organized structure. Moreover, it has been postulated that FA may reflect the degree of myelination and/or fiber bundle density.[4] NON participants had higher FA values and thus potentially greater WM organization and degree of connectivity. Therefore, it is possible that NON participants were able to more easily recruit additional neural resources or

better coordinate activity between regions, allowing them to remain effectively engaged in the visual-motor task (see Drummond for a formulation of the compensatory hypothesis).[11]

The VMC task is a relatively simple visual-motor task with a response timeout of 3.5 s; if participants are going to respond at all, they will do so within the time limit and will likely respond correctly. Therefore, changes in the VMC task associated with sleep deprivation probably reflect brief "attentional lapses."[12] Investigators have argued that the impact of sleep deprivation on attention takes place through multiple interacting brain systems, including "bottom-up" pressures from subcortical/brain stem regions modulating sleep-wake cycles and "top-down" efforts to remain alert in the face of sleep deprivation.[12,13] It is possible that increased cross-regional connectivity, as revealed by greater FA values, may help lessen these lapses by aiding top-down efforts to remain alert. It is not surprising that the differences in WM associated with vulnerability would be nonspecific to location; the diffuseness of these WM differences supports findings that an overall brain difference, rather than differences in more select regions, is likely to be associated with vulnerability to sleep deprivation.[2,13] This view is supported by the lack of sleep deprivation vulnerability findings for the decision-making tasks, which would likely require coordination of multiple cognitive/neural processes that may have differed between individuals (e.g., cognitive control, working memory capacity, processing speed, or ability to effectively learn novel decision-making tasks). Therefore, as argued by other researchers,[12] cognitive vulnerability to sleep deprivation may be best captured by a lower level task that emphasizes primary attention. Our findings demonstrate for the first time that disruptions in lower level attention associated with sleep deprivation can be tied to differences in brain structure.

A number of drawbacks to the current study should be noted. First is the skewed gender ratio and relatively restricted age range. Though there were no vulnerability group differences between the male and female participants, external validity would be bolstered with a larger sample of females and a broader range of ages. Secondly, it should be noted that prior sleep history was not collected for the participants, and sleep deprivation runs were not counterbalanced between sleep deprivation and rested conditions. Despite this, the contribution to the current results would likely be minimal, as West Point cadets live a highly regimented lifestyle that would result in similar pre-experimental sleep histories, and all subjects performed at 100% accuracy on the VMC task; thus there was no room for additional practice effects that could be introduced by the fixed run order. Nevertheless, future replications of this finding that include a more complete age and sex range as well as a more thorough assessment of prior sleep history are critically needed.

From the present study, we conclude that it is possible that more extensive development of cortical communication pathways contribute to a person's ability to function effectively when sleep deprived. This development reflected in the FA values likely continues through the lifespan of our participants, but the source of individual differences in development remains unknown. Further research will be required to clarify the effect greater WM connectivity has on cognitive and neural functioning under conditions of sleep deprivation.

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DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

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METHODS

The high-resolution T1 and diffusion tensor MR imaging (DTI)data analyzed here were acquired concurrently with an functional magnetic resonance imaging (fMRI)study examining the effects of sleep deprivation on decision making, the results of which are presented elsewhere.[1] The T1 and DTI data were collected only on Day 1 before and after the decision-making task. Stimuli for the task were presented using a PC notebook computer running Matlab with Psychophysics Toolbox extensions,[2,3] and projected with an LCD projection system onto a back projection screen located in the bore of the MRI and viewed by the participant through a mirror mounted on the top of the head coil. The responses were collected using the participant's right hand and an MR compatible 2-button response box.

Paradigm

The visual-motor control (VMC) task served as a control condition in the fMRI decision-making study. It was interspersed with 3 other conditions in an alternating block design across 4 runs. The three decision-making tasks were an alternative forced-choice with 2 choices (2AFC), an alternative forced-choice with 3 choices (3AFC), and an integrated decision-making task (ID). These tasks utilized novel abstract shapes adapted from Slotnick and Schacter,[4] which were created to reflect multiple exemplars of specific target stimuli. The matching nature of these exemplars served as the basis for the decision-making task where participants were asked to make judgments about whether test stimuli matched a target. For the 2AFC task, participants were shown a target shape and 2 other test shapes simultaneously below this target and asked "Which one?" (Figure S1a). Their task was to indicate which of the 2 test shapes best matched the target shape by pressing a button with their right hand. The 3AFC task was the same, but with 3 test shapes under the target rather than 2 (see Figure S1b). For both the 2AFC and 3AFC tasks there was only one correct answer. 2AFC and 3AFC task-types were presented on alternating runs, such that each condition appeared on a total of 2 of the 4 total runs. There were a total of 5 blocks per run, and there were six 2AFC or 3AFC trials per block for a total of 30 trials per run, and 2 runs for each per day for a total of 60 trials per day for each task.

The ID task followed the same format as the 2AFC task by presenting a target shape with 2 test shapes beneath, and the prompt "Exactly one?" (see Figure S1c). For this task, participants were asked to indicate whether one and only one of the test shapes matched the target shape. The nature of this task required that participants evaluate each shape and then "integrate" those individual evaluations into a final response. There were 3 possible cases—only one test shape matched (the answer would be *yes*), or both or neither test shapes matched (the answer would be *no*). There were ID trials on all 4 runs and 6 ID trials per block, for a total of 30 trials per run, and 4 runs per day for a total of 120 ID trials per day.

Interspersed throughout all 4 decision-making blocks was the VMC task, where participants were asked to indicate with a button press on which side an 'X' was presented (see Figure S1d). This was a simple, low-level visual-motor task in which participants generally performed at a high level of accuracy. A block of 8 VMC trials was presented between each decision-making task. Again, there were a total of 5 blocks per run for a total of 40 VMC trials per run and 4 runs per day, for a total of 160 VMC trials per day. Each individual trial, both decision-making and VMC, had a timeout of 3.5 s, but total block time remained fixed (interstimulus interval varied depending on individual response time). Each of the 4 runs took approximately 6.5 min, with a total of 26 min to complete the entire task. The order of task blocks was counterbalanced within- and between-subjects each day.

Analysis

Changes In Behavioral Performance Associated With Sleep Deprivation

For each of the conditions a *vulnerability score* was calculated ([Day 2 Task accuracy - Day 1 Task accuracy] / Day 1 Task accuracy). For this calculation, any run that resulted in greater than 20% missed trials was excluded from the final analysis and when more than 2 runs were excluded that participant was dropped from the final analysis. For the VMC change score, accuracy was calculated including missed trials (raw accuracy) while for the decision-making trials, timeout trials were excluded from the accuracy calculation (net accuracy). In this way, the VMC task became a simple measure of a participant's ability to maintain task attention in the face of sleep deprivation, since participants were usually highly accurate when they responded. By contrast, with missed trials excluded (and thereby the effect of attentional lapsing captured by the VMC task reduced), the accuracy on the decision-making tasks reflected the "higher order" cognitive functions that were required to accurately classify the stimuli (AFC tasks) and additionally, in the case of the ID task, maintain decisions in working memory and integrate them into a final output decision.

The results for the VMC task are reported in the body of the main text. In addition, we examined whether there was differential performance between groups across the 4 blocks on Day 2. As some participants were missing one run on Day 2 (i.e.,

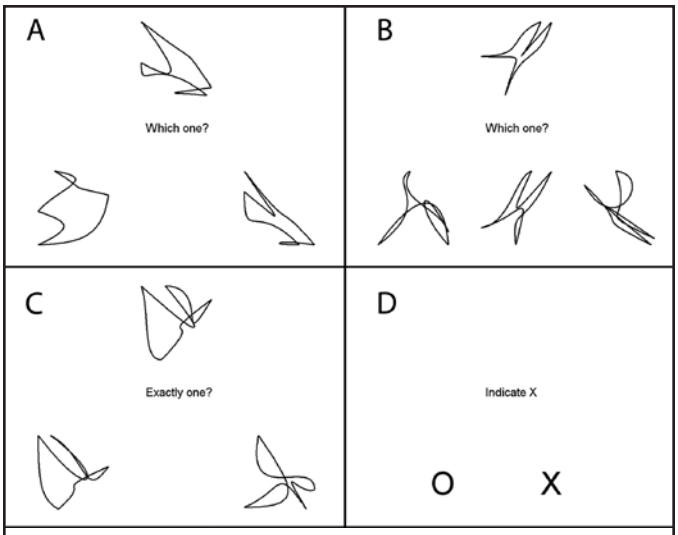

Figure S1—*a:* Example of the alternative forced-choice with 2 choices (2AFC) task. *b:* Example of the alternative forced-choice with 3 choices (3AFC) task. *c:* Example of the integrated decision-making (ID) task. *d:* Example of the visual-motor control (VMC) task.

failed to meet performance criteria of <20% missed trials), this issue was examined in 2 different ways. First, Day 2 runs 1 and 2 were averaged into a first half score, and runs 3 and 4 were averaged into a second half score. Mean performance for each subject was then examined in a 2 × 2 repeated measures ANOVA with run as within-subjects factors and group as a between-subjects factor. The ANOVA revealed a significant main effect of group ($F_{1, 22} = 39.36$, $P < 0.001$), but no significant effect of run or group by run interaction (all $F < 1$). The second way in which this was examined was to mean substitute performance scores for the missing runs. This analysis also failed to demonstrate a significant group by run interaction ($F_{3, 66} = 1.05$, *ns*). These results indicated that the decline in performance seen in the group more vulnerable to sleep deprivation (VUL) relative to the group less vulnerable to sleep deprivation (NON) was consistent across all 4 tasks blocks on Day 2.

Percent change scores were also calculated for the 2AFC, 3AFC, and ID tasks. Reflecting the fact that change on these tasks captured a different aspect of sleep deprivation than the VMC task, neither the 2AFC nor 3AFC percent change score correlated significantly with the percent change on the VMC task (Pearson r = −0.01 and −0.07 respectively, both *ns*). The correlation between the VMC and ID task only trended towards significance (Pearson r = 0.37, P < 0.09). The mean accuracy for each task on Day 1 and Day 2 as well as the mean percent change scores for the median splits on each task are presented in Table S1 of this supplement. Because there were no significant white matter (WM) differences identified by performance declines upon analysis in FMRIB Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl) for the 2AFC, 3AFC, and ID tasks, no further behavioral analysis was performed.

Table S1—Mean Accuracy for Day 1 and Day 2 by Task and Group as Well as the Mean Percent Change in Accuracy from Day 1 to Day 2

Task	Group	Mean Acc, Day 1	Mean Acc, Day 2	Mean % Change Acc
VMC	VUL	1.00	0.92	−0.08
	NON	1.00	0.98	−0.02
2AFC	VUL	0.91	0.84	−0.08
	NON	0.83	0.90	0.10
3AFC	VUL	0.78	0.71	−0.09
	NON	0.74	0.81	0.08
ID	VUL	0.69	0.64	−0.08
	NON	0.63	0.68	0.07

Structural Imaging Analysis

DTI analysis All image analysis was conducted with FSL. For the 25 direction diffusion-weighted images, motion and eddy current distortion were corrected with FMRIB's Diffusion Toolbox (FDT). After this, the 3 principle diffusion tensors for each voxel were calculated; from these tensors, fractional anisotropy (FA) and radial and axial diffusivity were computed on a voxel-by-voxel basis. Individual FA maps were then entered in the TractBased Spatial Statistics (TBSS) pipeline. This approach utilizes both affine and nonlinear transformations to align individual FA maps to FMRIB58_FA standard space, a high-resolution average of 58 FA images from healthy male and female subjects between 20 and 50 years of age, and MNI-152 standard space. These individual standard space FA images were then averaged and the resulting group mean FA image was skeletonized. Skeletonization consists of first thresholding the mean FA map to retain only values above 0.2. This technique ensured that only WM voxels were used. For each subject's registered FA image, the maximum FA value perpendicular to each voxel of the "standard" skeleton was retained and projected onto this skeleton to form a skeleton FA map for each individual participant. The individual registration vectors obtained during the above-described process were also applied to the mean diffusivity data in order to register these images into a common space.

Whole brain differences in FA and diffusivity between VUL and NON groups were analyzed on a voxel-by-voxel basis using FSL's *randomize* (which combines general linear model testing with permutation inference statistics). The threshold-free cluster enhancement (TFCE) option in FSL was used to correct for multiple comparisons across voxels and set at an α level of $P < 0.01$.[5] The results of the whole brain FA examination are presented in the main text. Only vulnerability groups defined using the VMC percent change score demonstrated significant pathway differences and this was only for the contrast of NON > VUL. Groups defined with the 3 decision-making tasks—2AFC, 3AFC, and ID—did not show any significant pathway differences at $P < 0.01$ or at the more liberal threshold of $P < 0.05$.

Pathway of interest (POI) analysis was performed to accomplish two goals: (1) test the visually apparent laterality of NON > VUL contrast, and (2) test for linear correlations between the percent change VMC score and FA values across the entire group. POIs were defined by converting specific WM regions in the Johns Hopkins University (JHU) DTI-based atlas available in FSL into masks (see Figure S2 for examples of the pathway masks used for this analysis). Masks were calculated from these regions by first thresholding to include voxels with

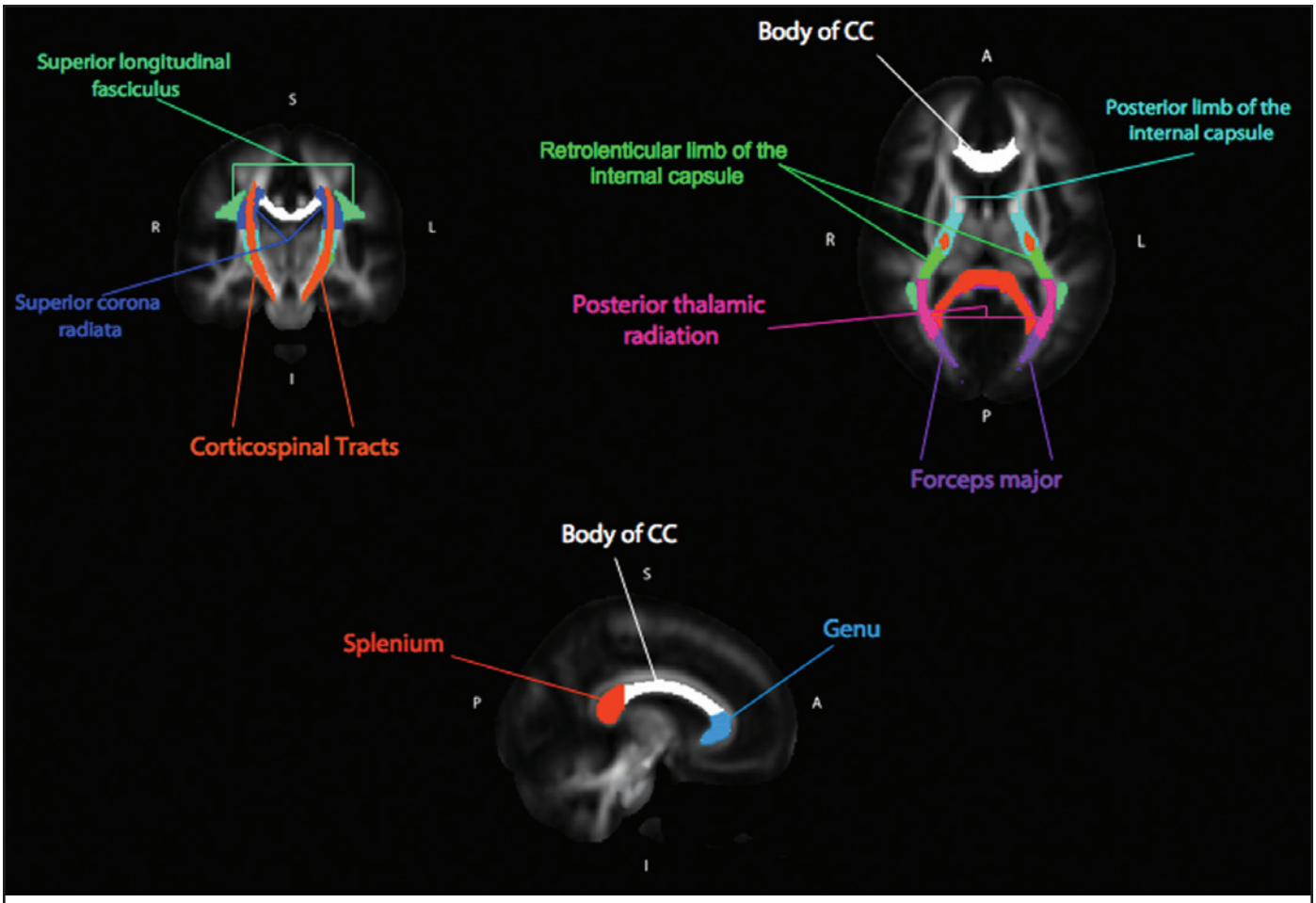

Figure S2—Clockwise from left is one coronal, one axial, and one sagittal brain slice with the Johns Hopkins University (JHU) atlas used to extract fractional anisotropy (FA) and mean diffusivity (MD) values.

25% or greater certainty of being within a specific POI and then converted to binary values of 1 within the POI and 0 elsewhere. Binary POIs were then multiplied against each individual participant's standard space FA skeleton to extract the mean FA value contained within each POI. FA values included all voxels within the defined pathway, not just those for which there were statistical differences revealed by whole brain comparison.

Diffusivity Analysis

We examined whether the groups identified with the VMC task showed any differences in mean diffusivity (D) across the pathways identified in the whole brain FA analysis. Mean diffusivity (MD) values within the specific pathways identified by the whole-brain FA analysis were extracted in the same way as FA values and tested in a 2 × 2 × 11 repeated measures ANOVA with hemisphere and region as within-subjects factors and group as a between-subjects factor. The results of this analysis indicated only a marginal effect of group ($F_{1, 22} = 3.61$, $P < 0.08$), with no significant group interactions. There was a significant main effect of hemisphere ($F_{1, 22} = 19.96$, $P < 0.001$), region ($F_{10, 22} = 168.70$, $P < 0.001$), and a hemisphere by region interaction ($F_{10, 22} = 8.17$, $P < 0.001$). These results indicate that D was less sensitive to the relationship between sleep deprivation and cognition than FA.

WM Volumetric Analysis

To complement the DTI analysis, we examined whether WM volume plays a role in the FA differences between VUL and NON groups. This was accomplished in 2 ways—first, total WM volume was entered as a covariate in the POI analysis (see main text), and secondly, specifically parcellated regions of WM where there were corresponding FA differences were directly tested for volumetric differences between groups (see below). For these analyses, the T1 images were processed using the FreeSurfer software package (http://surfer.nmr.mgh.harvard.edu). Two T1 images were collected, motion corrected, and averaged. Then all non-brain structures were removed, intensity was normalized, WM was isolated from grey matter based on intensity, and topological deformations were fixed and the grey matter surface area smoothed. Finally, subcortical regions were segmented from the overall grey and WM assignments. This method results in a total cerebral WM measure that was subsequently used in the covariance analysis contained in the main text.

In addition to whole brain WM volume, we examined specific regions of WM corresponding to the regions of FA where there were significant differences between VUL and NON groups. To begin, FreeSurfer was used to parcellate the grey matter into specific cortical regions using an algorithm based on a probabi-

listic atlas and a Bayesian classification rule to assign a neuroanatomical label to each voxel in the reconstructed image. This technique has been shown to be accurate and reliable in normal brains.[6] Using this procedure, measurements of volume were calculated automatically from neural structures and labeled as they corresponded to the predefined atlas.[7] This cortical parcellation serves as the basis of another algorithm that assigns WM labels to those regions of WM underneath each cortical label. WM labeling was limited to a 5-mm distance to avoid the centrum semiovale and periventricular regions. The result of this process is that there is one labeled WM area corresponding to each parcellated cortical area.

In order to examine whether there was, in addition to differences identified in FA values, a relationship between the volumes of specific regions of WM and vulnerability, parcellated regions of WM from the T1-weighted images were tested. Independent *t*-tests were conducted for ROIs that corresponded to regions where there were significant differences in FA values. There were no significant differences in WM volume for any of the ROIs: corpus callosum, $t_{22} = 0.09$, P = 0.929; right precuneus, $t_{22} = -0.08$, P = 0.937; left precuneus, $t_{22} = -0.26$ P = 0.796; right lingual, $t_{22} = -0.88$, P = 0.391; and left lingual, $t_{22} = -0.07$, P = 0.946. These results demonstrate that even when focused on specific regions of WM where there were FA differences between groups, these effects could not be attributed to volumetric differences.

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