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# White matter structure in older adults moderates the benefit of sleep spindles on motor memory consolidation

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# White matter structure in older adults moderates the benefit of sleep spindles on motor memory consolidation

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## Abstract

Sleep spindles promote the consolidation of motor skill memory in young adults. Older adults, however, exhibit impoverished sleep-dependent motor memory consolidation. The underlying pathophysiological mechanism(s) explaining why motor memory consolidation in older adults fails to benefit from sleep remains unclear. Here, we demonstrate that male and female older adults show impoverished overnight motor skill memory consolidation, relative to young adults, with the extent of impairment being associated with the degree of reduced frontal fast sleep spindle density. The magnitude of the loss of frontal fast sleep spindles in older adults was predicted by the degree of reduced white matter integrity throughout multiple white matter tracts known to connect subcortical and cortical brain regions. We further demonstrate that the structural integrity of selective white matter fiber tracts, specifically within right posterior corona radiata, right tapetum, and bilateral corpus callosum, statistically moderates whether or not sleep spindles promoted overnight consolidation of motor skill memory. Therefore, white matter integrity within tracts known to connect cortical sensori-motor control regions dictates the functional influence of sleep spindles on motor skill memory consolidation in the elderly. The deterioration of white matter fiber tracts associated with human brain aging thus appears to be one pathophysiological mechanism influencing subcortical-cortical propagation of sleep spindles, and their related memory benefits.

## Significance Statement

Numerous studies have shown that sleep spindle expression is reduced and sleep-dependent motor memory is impaired in older adults. However, the mechanisms underlying these alterations have remained unknown. The present study reveals that age-related degeneration of white matter within select fiber tracts is associated with reduced sleep spindles in older adults. We further demonstrate that, within these same fiber tracts, the degree of degeneration determines whether or not sleep spindles can promote motor memory consolidation. Therefore, white matter integrity in the human brain, more than age per se, determines the magnitude of decline in sleep spindles in later life, and with it, the success (or lack thereof) of sleep-dependent motor memory consolidation in older adults.

## Introduction

An established literature in young adults demonstrates that sleep, relative to wakefulness, preferentially supports the offline consolidation of motor sequence memory, affording stability that slows forgetting (Smith and MacNeill, 1994; Fischer et al., 2002; Walker et al., 2002; Korman et al., 2003; Walker et al., 2003b; Robertson et al., 2004; Fischer et al., 2005; Walker et al., 2005; Spencer et al., 2006; Nishida and Walker, 2007; Spencer et al., 2007; Rickard et al., 2008; Barakat et al., 2011; Tucker et al., 2011; Wilson et al., 2012; Fogel et al., 2013; Nettersheim et al., 2015). Consistent with previous reports (though see, (Karni et al., 1994; Fischer et al., 2002), these overnight benefits in motor skill consolidation correlate with 1) the amount of NREM sleep or specifically stage 2 NREM sleep, particularly in the last quartile (Smith and MacNeill, 1994; Walker et al., 2002; Walker et al., 2003; Robertson et al., 2004; Nishida and Walker, 2007; Barakat et al., 2011; Fogel et al., 2013), and 2) the fast-frequency sleep spindles that predominate during these stages (Nishida and Walker, 2007; Barakat et al., 2011; Fogel et al., 2013). The latter finding is of particular relevance considering a recent report establishing the causal role of stage 2 NREM fast frequency sleep spindles in actively promoting motor skill memory consolidation (Lustenberger et al., 2016). This sleep spindle-dependent consolidation effect is associated with a network-level reorganization of motor memories throughout primary and secondary motor cortices, sensorimotor parietal cortex, basal ganglia, and cerebellum (Fischer et al., 2005; Walker et al., 2005; Fogel et al., 2013).

In contrast to young adults, multiple studies have demonstrated reduced sleep spindles (De Gennaro and Ferrara, 2003; Martin et al., 2013; Mander et al., 2014) and impoverished overnight motor skill memory consolidation in older adults, resulting in nominal retention differences relative to equivalent time periods spent awake (Spencer et al., 2007; Wilson et al.,

2012; Fogel et al., 2013). Moreover, sleep spindles do not furnish the same functional reorganization of motor memories observed in young adults (Fogel et al., 2013). While these findings reveal impairments in sleep-dependent motor memory consolidation processes, they do not explain why sleep spindles fail to transact a consolidation benefit to motor skill memories in older adults.

One candidate mechanism explaining the failure of sleep-dependent motor memory consolidation is age-related deterioration in the structural integrity of white matter in the brain. Major commissural and projection white matter fiber tracts support thalamocortical communication between ipsilateral and contralateral sensorimotor regions and subcortical striatal and hippocampal regions that are integral to motor function (Schmahmann et al., 2008). Moreover, integrity of these same tracts is necessary for learning and retention of skilled motor memory routines. For example, blocking myelination within the corpus callosum white matter tract following novel motor learning in rodents abolishes the subsequent long-term consolidation of those motor skill memories (McKenzie et al., 2014). Moreover, greater time devoted to motor skill learning results in higher corpus callosum white matter integrity (Bengtsson et al., 2005). Additionally, white matter integrity in these commissural fiber tracts is significantly degraded in older adults (Salat et al., 2005), with the degree of degradation predicting the severity of cognitive and motor deficits (Ryberg et al., 2011).

White matter structure within these circuits is not only linked to motor skills. Importantly, white matter integrity within cortico-subcortical tracts that encompass the corpus callosum predict the quantitative and qualitative features of sleep spindles in young adults (Piantoni et al., 2013)—loops that are causally necessary for the expression of sleep spindles (Steriade et al., 1987). Given that white matter regulates the expression of sleep spindle

oscillations, it is therefore reasonable to posit that the structural integrity of these pathways also dictates the functional benefits associated with sleep spindles, including motor memory consolidation (Fischer et al., 2005; Walker et al., 2005; Fogel et al., 2013).

Building on this evidence, we tested the hypothesis that the severity of white matter atrophy predicts the severity of sleep spindle reduction in older relative to young adults. We further hypothesize that degeneration of specific portions of white matter, such as regions of the corpus callosum linking sensorimotor cortices, would additionally reduce effectiveness of sleep spindles in promoting overnight motor memory consolidation. We examined these hypotheses by combining overnight polysomnography (PSG) recording with full head EEG, high-resolution structural and diffusion tensor imaging (DTI), and assessment of performance on a validated motor skill task (MST) in young and older adults.

## Materials and Methods

Ninety-one community-dwelling, healthy participants, recruited through local advertisements, completed the study (**Table 1)**, with thirty-one healthy older adults (22 females, mean±s.d., 73.5±5.2 years) and twenty healthy young adults (12 females, mean±s.d., 20.4±2.0 years) training on a validated motor skill task (MST) in the evening and retesting in the morning after sleep 10-12 hours later. Twenty healthy older adults (17 females, mean±s.d., 74.1±7.1 years) and twenty healthy young adults (9 females, mean±s.d., 21.7±2.9 years) acted as wake controls, training in the morning and retesting 10-12 hours later in the evening. The study was approved by the local human studies committee at University of California, Berkeley, with all participants providing written informed consent. Exclusion criteria included presence of

neurologic, psychiatric or sleep disorders, current use of antidepressant or hypnotic medications, or being left handed. Participants were free of depressive symptoms (Yesavage et al., 1982), and all scored >25 on the mini mental state exam (Folstein et al., 1975). Furthermore, in addition to neuroradiological assessments and medical interviews (cf. (Mander et al., 2013; Mander et al., 2014); obtained within one year of study entry), elderly participants performed within two standard deviations of their age-matched control group on tests of 1) episodic memory (Wechsler, 1987; Delis et al., 2000) and 2) frontal function (Reitan, 1958; Zec, 1986) (**Table 1**). Prior to study entry, older participants underwent sleep disorders screening with a polysomnography (PSG) recording night (described below) reviewed by a board certified sleep medicine specialist (author B.L.). Participants were excluded if they displayed evidence of a parasomnia or an Apnea/Hypopnea Index ≥15 (Young et al., 2002). All participants abstained from caffeine, alcohol, and daytime naps for the 48 hr before and during the study. Participants kept normal, habitual sleep-wake rhythms and averaged 7–9 hr of reported time in bed per night prior to study participation, as verified by sleep logs (**Table 1**).

***Experimental Design and Statistical Analyses***

Young and older participants in the sleep groups entered the lab in the evening of the experimental night and were trained on a validated motor skill task (MST; described below). Following training, participants were given an 8 hour sleep opportunity, measured with PSG, starting at their habitual bed time, based on sleep logs 5 days before the study date (**Table 1**). Polysomnography recording included a 19-channel EEG array (details below). Approximately 2 hours post-awakening, participants underwent high-resolution structural MRI scanning and diffusion tensor imaging (DTI), followed by the MST retest.

Young and older participants in the wake groups entered the lab in the morning and were trained on a MST sequence, returning 10-12 hours later for the MST retest. Importantly, all wake group participants were instructed not to nap during this period, and no participant reported napping during this interval.

To assess MST performance differences between groups, two three-way repeated measures ANOVAs were used to compare MST speed (number of correct sequences typed in each 30 s trial) and accuracy (error rate in each 30 s trial) between young and older adults, with age group (young/older) and sleep condition group (sleep/wake) as between subjects factors and trial (baseline: trials 1 & 2 mean, post-training: trials 11 & 12 mean, retest: trials 14-16 mean) as a within subjects factor. This ANOVA was followed by 8 *post hoc* tests comparing the difference between baseline and post-training (initial learning) and post-training and retest (motor skill memory consolidation) between sleep condition and age groups employing false discovery rate (FDR) correction for multiple comparisons (Benjamini and Hochberg, 1995). Effect sizes associated with the impact of age on offline motor skill memory consolidation were statistically compared across sleep and wake condition groups to determine if the relative degree of age-related impairment depended on the presence or absence of sleep. Assessing the impact of motor sequence on MST speed and accuracy during initial learning (baseline—post-training trials) and motor skill memory consolidation (post-training—retest trials), independent samples t-tests were employed comparing between sequence A and B utilizing FDR correction for multiple comparisons. The same approach was used to compare neuropsychological status variables between sleep and wake condition groups in older adults. If differences were detected, these variables were correlated with MST measures of initial learning and motor skill memory

consolidation to determine if differences in neuropsychological status or motor sequence impacted MST performance across trials.

Age effects, defined as the difference between young and older adults, were examined in sleep variables using ANOVA models. Specifically, differences in sleep staging variables between age groups were compared using independent samples t-tests, employing the FDR correction for multiple comparisons (Benjamini and Hochberg, 1995). Stage 2 NREM fast sleep spindle density across the whole night were compared between age groups, with FDR correction utilized across all 19 EEG derivations. Differences in Stage 2 NREM fast sleep spindle density were further examined by employing a three-way repeated measures ANOVA, with age group as a between subjects factor and quartile (1-4) and EEG derivation (1-19) as within subjects factors. Age effects at each electrode in each quartile were examined using post hoc tests, FDR corrected across 76 comparisons (Benjamini and Hochberg, 1995).

Multiple regression analyses were employed to examine associations between motor skill memory consolidation and stage 2 NREM sleep spindle density across the whole night and during the fourth quartile only, with FDR correction across all 19 channels. The correlation between motor skill memory consolidation and stage 2 NREM sleep spindle density at the EEG derivation demonstrating peak significance (CZ during fourth quartile) across all participants was then examined separately for young and older adults to determine if both groups exhibited similar motor skill memory and sleep associations. The separate correlations for each group were not examined at other derivations, and for this reasons *P*-values were not corrected across the 19-channel EEG array. To determine specificity of spindle and motor skill memory consolidation effects, fast sleep spindle density was also correlated with initial learning, and motor skill memory consolidation was also correlated with sleep stage measures and subjective sleepiness

variables. Finally, motor skill memory consolidation was also associated with fast sleep spindle density during all NREM sleep and NREM slow wave sleep, to determine if these associations were specific to sleep spindles during stage 2 NREM sleep.

DTI data were analyzed using FSL 5.0 to compare white matter mean diffusivity between young and older adults. As described in the DTI analysis section, an independent samples t-test was used to determine the degree to which MD values were higher in older relative to young adults at a voxelwise, FWE-corrected level. An ANCOVA model with age group as a between subjects factor and fourth quartile stage 2 NREM sleep fast sleep spindle density as a covariate was utilized identify which white matter regions were associated with fast sleep spindles even when controlling for the effects of age group. Thus, incorporating age group as a factor in the analysis determines whether or not effects are driven by large age group differences, averting spurious correlations.

Mean MD values across significant clusters were then extracted and compared to determine if white matter MD in these regions differed between young and older adults. To determine whether white matter associations with fast sleep spindles were linked to white matter degeneration in older adults, WML volume was correlated with mean sleep spindle-associated MD cluster values (nonparametric Kendall's $\tau$ correlation to accommodate the typical nature of WML data).

Moderation analyses were performed to determine whether the relationship between sleep spindles and motor skill performance was influenced by white matter brain structure. Here, moderation defines the interaction term which reflects the influence that white matter integrity has on dictating the strength of association between sleep spindles and motor skill memory

consolidation. The moderation analysis therefore investigated the influence of white matter MD values on the ability of CZ fast sleep spindles to predict motor skill memory consolidation. Age group, CZ fast sleep spindles, and associated MD cluster values were included as additional regressors in the multiple regression model. White matter tract regional specificity was determined by parcellation of twenty anatomically defined subregions from the validated JHU white matter tractography atlas (Wakana et al., 2004; Huang et al., 2005; Hua et al., 2008; Mori et al., 2008). FDR correction was applied to adjust for the number of moderation models examined after age correction.

All analyses were completed using SPSS version 24.0 (SPSS, Inc., Chicago, IL) and R version 3.3.2 (R Core Team, 2013).

***Motor Skill Task***

The MST was chosen because of its sensitivity to sleep (Walker et al., 2002; Walker et al., 2003a; Walker et al., 2003b; Kuriyama et al., 2004; Walker et al., 2005; Nishida and Walker, 2007; Tucker et al., 2011). The task required subjects to press four numeric keys on a standard computer keyboard with the fingers of their left (non-dominant) hand, repeating a five element sequence (4-1-3-2-4, sequence A) or (2-3-1-4-2, sequence B), "as quickly and as accurately as possible" for a period of 30 s. Sleep group participants learned one unique sequence in the evening before sleep, with the sequence (A or B) counterbalanced in session assignment across participants. The young and older adult wake group participants learned one unique sequence in the morning following sleep, with the sequence (A or B) counter-balanced across young and older adult group participants. The numeric sequence was displayed at the top of the screen at all times to exclude any working memory component to the task. Each key press produced a white

dot on the screen, forming a row from left to right, rather than the number itself, so as not to provide accuracy feedback. The computer recorded the key press responses, and each 30 s trial was automatically scored for the number of complete sequences achieved (speed) and the number of errors made (accuracy).

The encoding session consisted of twelve 30 s trials with 30 s rest periods between trials, and lasted a total of 12 min, and the retest session consisted of four trials, lasting 4 minutes. The efficiency of learning during training was measured by the subtracted difference between average performance (speed and accuracy, separately) at baseline (mean of trials 1-2) from the averaged performance post-training (mean of trials 11-12) (Walker et al., 2002). Consistent with previous reports in older adults, offline consolidation was determined by the subtracted difference between average performance post-training from the average performance at retest (mean of trials 14-16). The first retest trials were excluded due to the recognized 'warm up' effect that, if considered, may obscure offline improvement in older adults (Tucker et al., 2011). Finally, to adjust for group differences in overall number of typed sequences, associated with general slowing of reaction time in older adults on this task (Spencer et al., 2007; Tucker et al., 2011; Wilson et al., 2012; Pace-Schott and Spencer, 2013), differences across training and session were converted to percent change from the first two trials, in the case of training, or from the last two training session trials, in the case of sleep/wake session effects. MST data from two older adult participants was lost, leaving 29 older adults in the sleep group for all pertinent MST analyses.

***MRI scanning***

Scanning was performed on a Siemens Trio 3 Tesla scanner at the Henry H. Wheeler, Jr. Brain Imaging Center (BIC) equipped with a 32-channel head coil. Two high-resolution T1-weighted anatomical images were acquired using a 3D MPRAGE protocol with the following parameters: repetition time (TR), 1900 ms; echo time (TE), 2.52 ms; inversion time (TI), 900 ms, non-selective inversion pulse; flip angle, 9°; field of view (FOV), 256 mm; matrix, 256 × 256; 176 1.0 mm isometric sagittal slices.

Additionally, high-resolution, whole-head diffusion tensor imaging (DTI) data were acquired using a diffusion-weighted spin echo-planar imaging (EPI) method [time repetition (TR)/ time echo (TE) 7900/102 ms; 1 average; FatSat, echo spacing 0.83 ms; FOV 282×282 mm; matrix 128×128; 55 2.2 mm isometric axial slices; 9:45 scanning duration] employing parallel imaging reconstruction (GRAPPA) with acceleration factor 2 and 6/8 partial fourier in the phase encoding dimension. Diffusion-weighting along 64 separate directions was applied with a b value of 1000 s/mm$^2$, and 6 images without diffusion-weighting (b = 0 s/mm$^2$) were also acquired to aid preprocessing.

Finally, in older adults only, a high-resolution T2-weighted fluid-attenuated inversion recovery (FLAIR) image designed to enhance white matter hyperintensity (WMH) segmentation (Jack et al., 2001) was acquired with the following parameters: repetition time (TR), 6000 ms; echo time (TE), 388 ms; inversion time (TI), 2100 ms, non-selective inversion pulse; flip angle, 120°; field of view (FOV), 256 mm; matrix, 256 × 258; 160 1.0 mm isometric sagittal slices. This scan was not acquired in young adults, since it is unlikely healthy young adults, free of a history of neurological disorders, strokes, and traumatic brain injury would have white matter lesions.

***DTI analysis***

DTI data underwent standard preprocessing using the FSL 5.0 processing pipeline (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki) (Smith et al., 2004). First, the brain was extracted using the brain extraction toolbox (BET) (Smith, 2002), with an image without diffusion weighting (b = 0 s/mm$^2$) used for all registrations due to its greater structural contrast. Images were corrected for motion and eddy current distortion. Fractional anisotropy (FA) and Mean diffusivity (MD) maps were then estimated for each participant by using the FMRIB diffusion toolbox (FDT) to fit a tensor model to the corrected diffusion data. FA and MD maps were registered to standard template space using the nonlinear registration tool FNIRT. Next, a mean FA image was created and a threshold value of 0.2 was used to restrict analyses to voxels consistently identified as white matter. This image was then 'skeletonised' to centers of tracts common across all participants. Registered MD maps were then projected onto this skeleton, with the resulting data used for voxelwise whole brain analysis using the tract-based spatial statistics toolbox (TBSS) (Smith et al., 2006). An independent samples t-test was used to determine the effects of age on MD measures, and an ANCOVA model was employed to determine where MD was correlated with frontal sleep spindles while controlling for age group.

Models were estimated using the 'randomise' tool in FSL with 5000 permutations. Clusters were considered significant using the threshold-free cluster enhancement (TFCE) method employing whole-brain family-wise error (FWE) correction. Regional labels for significant clusters in all TBSS analyses were obtained from the John Hopkins University (JHU) white matter tractography atlas (Wakana et al., 2004; Huang et al., 2005; Hua et al., 2008; Mori et al., 2008). Mean MD measures within significant clusters were extracted and used in the below described independent samples t-tests and regression analyses. Regional MD measures

were extracted and examined by creating masks of significant voxels within distinct anatomically-defined white matter regions using the JHU white matter tractography atlas (Wakana et al., 2004; Huang et al., 2005; Hua et al., 2008; Mori et al., 2008).

WML volumes—those associated with white matter hyperintensities observed in T2-weighted FLAIR images—were extracted from T2-weighted FLAIR images and quantified using the Quanta 2.0 software package, as described previously (DeCarli et al., 2005; Lockhart et al., 2014). As WML are not expected in healthy young adults, FLAIR images were only collected in older adults. Images were manually traced along the dura mater to remove the cerebellum, subcortical brain tissue, and non-brain tissue.

Images were then corrected for intensity non-uniformities, and modeled as a combination of two Gaussian probability functions, with these functions corresponding to grey and white matter brain tissue and CSF. These two probability functions were used to segment out brain tissue for WML analysis, with the two exterior brain tissue voxels eroded to minimize the influence of partial volume effects on WML detection. An automated algorithm was then used to detect WMLs, defined as voxels with signal intensity greater than 3.5 standard deviations above the mean of all brain voxels (DeCarli et al., 2005; Lockhart et al., 2014). Detected voxels were then visually inspected to remove artifacts. WMLs were considered for analysis only if they appeared on 3 consecutive slices, visible on all three orientations. WML volumes were then estimated relative to the total intracranial volume. Images from two participants were lost due to computer error, leaving only 29 older participants for this analysis. Due to the non-normal distribution of WML values, the nonparametric Kendall's $\tau$ coefficient was derived to examine the association between WML and DTI-derived MD values in older adults.

***Sleep monitoring and EEG analysis***

Polysomnography (PSG) on the experimental night was recorded using a Grass Technologies Comet XL system (Astro-Med, inc., West Warwick, RI), including 19-channel electroencephalography (EEG) placed using the 10-20 system, electrooculography (EOG) recorded at the right and left outer canthi (right superior; left inferior), and electromyography (EMG). Reference electrodes were recorded at both the left and right mastoid (A1, A2). Data were digitized at 400Hz, and stored unfiltered (recovered frequency range of 0.1–100 Hz), except for a 60-Hz notch filter. Sleep was scored using standard criteria (Rechtschaffen and Kales, 1968). Sleep monitoring on the screening night was recorded using a Grass Technologies AURA PSG Ambulatory system (Astro-Med, inc., West Warwick, RI), and additionally included nasal/oral airflow, abdominal and chest belts, and pulse oximetry.

***Sleep-spindle analysis:*** Prior to spindle analysis, each EEG channel was re-referenced to the average of the left and right mastoid, allowing for commonality of reference. Artifacts in the time series were removed by visual rejection. Following artifact-rejection, EEG was band-pass-filtered using a finite impulse response function, set between 12-15 Hz (as used previously (Eschenko et al., 2006; Ferrarelli et al., 2007)). Automatic sleep-spindle detection analysis was then implemented using an established algorithm as described previously (Ferrarelli et al., 2007; Mander et al., 2011; Mander et al., 2014). In short, the amplitude of the rectified signal from stage 2 NREM sleep was used as a unique time series, identifying amplitude fluctuations exceeding threshold values, with the lower and upper values set at two and eight times the average amplitude. The peak amplitude for each spindle was defined as the local maximum

above the threshold, with the beginning and end of the spindle defined as points immediately preceding or following this peak, when the amplitude of the time series dropped below the cut-off threshold. This method was utilized, in part, because it advantageously defines spindles based on deviations from the mean signal amplitude and thus is robust against global differences in EEG signal that may be expected when comparing across individuals (Ferrarelli et al., 2007).

The algorithm-determined spindles were restricted only to those events falling within the specified frequency range. Post-detection, sleep spindles were separated into slow (12-13.5Hz) and fast (13.5-15Hz) spindles for analysis; a separation consistent with the existence of two distinct peaks in sigma activity during sleep proximal to the threshold applied in the current study, with these distinct peaks expressing distinct topographical and developmental trajectories (De Gennaro and Ferrara, 2003). The combination of fast and slow spindle density represents total spindle density, with total spindle density values consistent with those reported in previous studies (De Gennaro and Ferrara, 2003). Given previous reports linking age effects on sleep spindles and sleep effects on motor skill learning may differ by quartile of Stage 2 NREM sleep (Walker et al., 2002; Martin et al., 2013), stage 2 NREM sleep was separated into quartiles and spindle density was calculated within each quartile. Sleep spindle density was used to predict motor learning effects, as per our *a priori* hypothesis.

***Measures of subjective sleepiness***

Subjective sleepiness was measured using a validated visual analog scale (Monk, 1989) collected every two hours throughout the study while subjects were awake, including at the beginning of each testing session. Subjective ratings were compared between testing sessions to

assess the change in subjective sleepiness and its association with motor learning between and across groups.

## Results

***Impact of age and sleep on motor skill learning and offline consolidation***

We first examined whether age group (young/old) or wake/sleep condition group influenced MST speed (number of correctly typed sequences per 30 s block) or accuracy (error rate per 30 s block) across testing trials (**Fig. 1A**). For MST speed, a three-way repeated measures ANOVA was conducted, with trial (baseline mean, post-training mean, retest mean) as a within subject factor, and age group (young/older), and wake/sleep condition group as between subjects factors. There were main effects of age group ($P<0.001$) and trial ($P<0.001$) and, importantly, both age group×trial ($P<0.001$) and wake/sleep condition group×trial interactions ($P=0.039$). No other significant main or interaction effects were detected, including age group×wake/sleep condition group ($P=0.343$) and age group×wake/sleep condition group×trial ($P=0.543$) interaction effects. These findings indicate that age impairs motor skill performance, while brain state (wake/sleep) significantly influences offline motor memory retention.

Next, we examined our hypothesis that older adults exhibit a less robust benefit from sleep on motor skill memory. These *post hoc* tests directly compared the performance change from baseline to post-training and post-training to retest across sleep and age groups. We employed a false discovery rate (FDR) correction for multiple comparisons (Benjamini and Hochberg, 1995); 8 comparisons, total; **Fig. 1B**).

There were no significant differences in initial learning acquisition, measured by the change in MST speed from baseline to post-training, across the four groups: i) young sleep condition group, ii) young wake condition group, iii) older sleep condition group, and iv) older wake condition group (all $P>0.1$ uncorrected, $P>0.2$ FDR corrected).

Importantly, however, there were significant differences in terms of subsequent offline degree of motor skill memory consolidation (post-training vs. retest, or delayed retention) between groups, across wake and sleep periods. As in prior studies (e.g. (Fischer et al., 2002; Walker et al., 2002; Spencer et al., 2006)), young adults show superior offline memory retention across sleep relative to young adults across wake ($P=0.005$, $P=0.017$ FDR corrected, cohen's d=0.94). In contrast, no such typical sleep-dependent wake/sleep benefit was observed in older adults ($P=0.260$, $P=0.347$ FDR corrected, cohen's d=0.33). A direct comparison demonstrated that older adults showed significantly less overnight motor skill memory retention benefit relative to young adults in the sleep condition groups ($P<0.0001$, $P<0.0001$ FDR corrected, cohen's d=1.7). However, young adults also exhibited less motor skill memory forgetting than older adults in the wake condition groups ($P=0.006$, $P=0.017$ FDR corrected, cohen's d=0.92). To determine if the impact of age on motor skill memory consolidation differed by sleep condition, the age group effect sizes in the sleep and wake condition groups were statistically compared. The size of the effect of age was significantly larger across sleep than across wake (cohen's d=1.7 *versus* 0.92, $P=0.049$), demonstrating that older adults show the greatest relative impairment in offline consolidation across periods that include sleep.

The same three-way repeated measures ANOVA of MST accuracy only revealed a main effect of trial ($P=0.004$), indicating that the impact of age and sleep condition groups on the change in MST performance across trials was specific to MST speed. Additionally, MST

sequence (A/B) did not influence MST performance change across trials (speed or accuracy; all independent sample t-tests comparing baseline to post-training and post-training to retest $P>0.08$ uncorrected, $P>0.18$ FDR corrected).

Together, these data support the experimental hypothesis that overnight motor memory consolidation is significantly diminished in older relative to young adults.

***Impact of age on fast sleep spindle density***

We next examined the impact of age on sleep, focusing *a priori* on fast sleep spindles (13.5-15Hz) during stage 2 NREM sleep, due to the recognized association between motor sequence consolidation and late night stage 2 NREM sleep and associate fast sleep spindles (Smith and MacNeill, 1994; Walker et al., 2003; Peters et al., 2008; Tamaki et al., 2009; Lustenberger et al., 2016) (see **Table 2** for age differences in sleep stage characteristics). Two-sample t-tests, FDR corrected for 19 comparisons, revealed that fast sleep spindle density during stage 2 NREM sleep was significantly lower in older relative to young adults, primarily in frontal and central EEG derivations (**Fig. 2,** peak age difference detected at FZ, CZ, and T4). Since the consolidation of motor skill memory may depend on stage 2 NREM sleep quartile (Walker et al., 2002), as well as the impact of age on fast sleep spindles (Martin et al., 2013), we next explored the impact of age group on fast sleep spindles across the sleep period.

A three-way repeated measures ANOVA with age group (young/older) as a between subjects factor and quartile of the stage 2 NREM sleep period (1st-4th) and EEG derivation (channels 1-19) as within subjects factors revealed 1) significant age group×EEG derivation ($P<0.001$) and age group×quartile×EEG derivation ($P=0.013$) interactions, 2) a significant main

effect of derivation ($P$<0.001), and 3) a trend of a main effect of age group ($P$=0.059) (**Fig. 2A**). That is, older adults exhibited a reduction in stage 2 NREM fast sleep spindles at specific EEG derivations with the degree of reduction depending on the quartile of the stage 2 NREM sleep period. Employing FDR correction for multiple comparisons (76 comparisons across four quartiles), we further identified that age effects (difference between young and older adults) centered on frontal and central EEG derivations. For raw differences, effect size, and significance, these age differences became more prominent as the night progressed, showing the largest differences in fast sleep spindle density during the fourth quartile (**Fig. 2A & B**).

***Aging, fast sleep spindles, and motor memory consolidation***

Next, we tested the hypothesis that stage 2 NREM fast sleep spindle density predicted the degree of overnight, offline motor skill memory consolidation. Across all participants, stage 2 NREM fast sleep spindles at multiple central, frontal, and temporal EEG derivations positively predicted success of overnight motor skill memory consolidation (**Fig. 3A**). Consistent with the impact of age on fast sleep spindles, FZ and CZ derivations demonstrated the most robust associations, remaining significant following FDR correction across the 19 electrode array (**Fig. 3B**; strongest at CZ; r=0.43, $P$=0.002). Since age-related reductions in stage 2 NREM fast sleep spindles were maximal in the fourth quartile, we then examined associations between stage 2 NREM fast sleep spindles in the fourth quartile and offline motor skill memory consolidation. Consistent with our hypothesis, fast sleep spindle and motor skill memory consolidation associations were 1) stronger in the fourth quartile than collapsed across all stage 2 NREM sleep (**Fig. 3C-D**), and consistent with the nature of the left-handed, uni-manual task, 2) also strongest

441 in the contra-lateral (to hand) right-hemisphere and midline derivations (Fp2, FZ, CZ, and T4),
442 all remaining significant following FDR correction (**Fig. 3C-D**; strongest at FZ and CZ).

443 Building on our planned comparisons between age groups, these significant midline
444 relationships were present in young adults (r=0.48 *P*=0.033), but not in older adults (r=0.08
445 *P*=0.679). Total sleep time, sleep efficiency, and subjective sleepiness did not predict overnight
446 motor memory consolidation effects for either young or older adults, or for all subjects combined
447 (all $r^2$<0.08, *P*>0.18). Performance improvement across initial training also did not correlate with
448 fast sleep spindles in either young, old, or all participants combined, *P*>0.143). Thus, fast sleep
449 spindles, specifically and selectively in the last quartile of stage 2 NREM sleep, were associated
450 with overnight motor skill memory retention in young but not older adults.

451 Beyond our *a priori* focus on stage 2 NREM sleep fast sleep spindles, we also examined
452 associations across all adults between motor skill memory consolidation and fast sleep spindle
453 density as well as the durations of total sleep time, REM sleep, slow wave sleep, and total
454 NREM sleep in post-hoc analyses. No significant associations were detected between motor skill
455 memory consolidation and total sleep time, time spent in slow wave sleep or overall NREM
456 sleep time (all *P*>0.07). Moreover, REM sleep time, percent of the sleep period spent in REM
457 sleep, and the time or percent of the sleep period spent in REM sleep during the fourth quartile
458 did not predict motor skill memory consolidation (all $r^2$<0.03 , all *P*>0.24). Critically, CZ fast
459 sleep spindles remained a significant predictor of motor skill memory consolidation when stage 2
460 NREM sleep metrics were included in the regression models for analyses collapsed across all
461 subjects (r=0.43, fourth quartile sleep fast sleep spindles at CZ *P*=0.002, fourth quartile stage 2
462 NREM sleep duration *P*=0.697) and in young subjects alone (r=0.59, fourth quartile sleep fast
463 sleep spindles at CZ *P*=0.037, fourth quartile stage 2 NREM sleep duration *P*=0.091).

Therefore, fast sleep spindles and not duration of REM and NREM sleep stages in general predicts the success of motor skill memory consolidation.

***Aging, white matter structure, and fast sleep spindles***

We next tested the experimental predictions that white matter degeneration in the brain—assessed using diffusion tensor imaging (DTI)—was greater in older relative to young adults and accounted for the age-related reduction in fast sleep spindles. We focused on white matter, because 1) sleep spindles are expressed in cortico-thalamic loops (Steriade et al., 1987; De Gennaro and Ferrara, 2003), 2) sleep spindle expression is associated with DTI white matter measures in young adults (Piantoni et al., 2013), and 3) white matter measures impact the long-term retention of motor skills (Bengtsson et al., 2005; McKenzie et al., 2014). Analyses examined mean diffusivity (MD), with *higher* MD values in white matter regions (i.e., greater diffusivity of water molecules within white matter tissue) potentially reflecting *lower* white matter integrity due to white matter degeneration. MD is commonly used to estimate the degree of 'white matter integrity'. However, without confirmation, differences in any DTI measure may merely reflect individual differences in the distribution of orientation of axons within white matter fiber tracts (Jones et al., 2013). To verify that MD measures reflected, in part, differences in white matter integrity, MD values were associated with white matter lesion (WML) volume derived from T2-FLAIR imaging acquired in older adults. Together, these analyses allowed us to evaluate whether age-related differences in white matter integrity tracked with changes in sleep spindle expression.

First, we compared white matter MD between young and older adult groups using an independent samples t-test, employing whole-brain FWE correction. Numerous white matter

fiber tracts displayed significantly higher white matter MD in older relative to young adults, reflecting, potentially, reduced white matter integrity due to age-related degeneration within white matter fiber tracts (**Fig. 4A**). Results were overlaid on the Johns Hopkins University (JHU) white matter atlas containing 48 distinct white matter regions to determine the specific anatomical tracts exhibiting age effects (Wakana et al., 2004; Huang et al., 2005; Mori et al., 2008).

An ANCOVA model, using age group and midline (CZ) fast sleep spindle density in the fourth quartile to predict MD with whole-brain FWE correction applied, demonstrated that commissural and projecting fiber tracts (**Fig. 4B; Table 3**), including the corpus callosum that has a well-recognized association with motor skill memory (McKenzie et al., 2014), were associated with fourth quartile fast sleep spindle density in both young and older adults. Specifically, higher MD in these regions was associated with a progressive diminution in fast sleep spindle density in the fourth quartile across all individuals combined (r=-0.67 $P$<0.001; **Fig. 4C)**, and in older (r=-0.59 $P$<0.001) and young (r=-0.59 $P$=0.006) adults separately (**Fig. 4C)**. Critically, all these significant clusters were detected in the previous age-group analysis, demonstrating that white matter MD predicted frontal fast sleep spindle density in regions demonstrating age effects (**Fig. 4C**).

Further verifying that MD differed by age in these voxels, MD in these white matter regions was significantly higher ($P$<0.001; **Fig. 4B**) while FA was significantly lower ($P$<0.001) in older relative to younger adults, potentially representing lower white matter integrity in older adults. Consistent with this interpretation, white matter lesion (WML) volume also correlated with mean MD values (Kendall's $\tau$ = 0.42, $P$=0.001; **Fig. 4D**). Therefore, the age difference in

MD in these fiber tracts likely reflects, in part, age-dependent erosion of white matter integrity, which results in reduced expression of late night stage 2 NREM frontal fast sleep spindles.

To determine whether the association between white matter MD values and fast sleep spindles in the last quartile of the night detected at CZ was specific, MD values were extracted from the significant voxels presented in Figure 4B. The values were then compared to fast sleep spindle density during the fourth quartile at 1) a posterior EEG derivation, which did not exhibit an age effect on fourth quartile fast sleep spindle density (at O1 $P$=0.267), and 2) at a frontal EEG derivation, which did exhibit an age effect (at F7 $P$=0.045 FDR corrected), yet did not show a significant sleep spindle effect on motor memory consolidation (at F7 $P$=0.238 uncorrected). Additionally, white matter MD values were included in multiple regression models predicting fourth quartile fast sleep spindle density at O1 and F7, while controlling for age group. These models revealed that, while white matter MD, averaged across all significant voxels, was associated with fourth quartile CZ fast sleep spindle density, MD in this cluster was not associated with fourth quartile O1 (for MD $P$=0.265, for Age $P$=0.119) or F7 (for MD $P$=0.350, for Age $P$=0.247) fast sleep spindle density. Therefore, white matter integrity in these select commissural and largely right hemisphere projecting fiber tracts, including the corpus callosum, corona radiata, and thalamic radiations (see **Table 3** for full list), specifically predicts fast sleep spindle density only at midline and right hemisphere EEG derivations exhibiting both an age effect and an effect on motor memory consolidation.

***Moderation of sleep spindle-related motor memory consolidation by white matter structure***

Finally, to test the hypothesis that the decline in white matter integrity, which was associated with a reduction in midline frontal fast sleep spindles, significantly contributed to

532 impairments in offline motor memory consolidation, moderation analyses were performed. If the
533 relationship between last quartile fast sleep spindles and overnight motor memory consolidation
534 is independent, then the inclusion of white matter MD (the moderator) into a statistical model
535 with frontal fast sleep spindles and motor memory retention should have no interacting influence.
536 However, if white matter integrity moderates the relationship between spindles and overnight
537 motor memory consolidation, white matter MD and sleep spindles should interact significantly.

538 Consistent with the latter prediction, the experimental hypothesis, there was a significant
539 moderation effect (interaction) between the mean MD within the white matter cluster reported in
540 Table 3 and midline fast sleep spindles (MD × CZ fast spindles; $P$=0.045). These findings
541 indicate that the efficacy of sleep spindles to support motor memory consolidation may depend
542 on the integrity of the white matter supporting their expression. However, when controlling for
543 age group, it was reduced to a trend for MD (MD×CZ sleep spindles $P$=0.106). Given this trend
544 after the inclusion of age group for MD, it is possible that only select white matter regions within
545 this cluster significantly account for the age effect on sleep spindle-dependent motor memory
546 consolidation. To determine the specific anatomical white matter regions moderating the
547 influence of sleep spindles on motor memory consolidation, after accounting for age, we utilized
548 the Johns Hopkins University (JHU) white matter atlas to parcellate the cluster predicting sleep
549 spindle density (see **Table 3**) into twenty distinct anatomical regions of interest (Wakana et al.,
550 2004; Huang et al., 2005; Mori et al., 2008). Of these twenty individual regions of interest, four
551 remained significant in demonstrating moderation when controlling for age group for MD,
552 including the body and splenium of the corpus callosum (for MD $P$=0.012, JHU-4; $P$=0.020,
553 JHU-5), the right posterior corona radiata (for MD $P$=0.040, JHU-25), and the right tapetum (for
554 MD $P$=0.020, JHU-47), all of which remained significant after FDR correction (for JHU-4

555 *P*=0.027 FDR corrected, for JHU-5 *P*=0.027 FDR corrected, for JHU-27 *P*=0.040 FDR
556 corrected, for JHU-47 *P*=0.027 FDR corrected; **Fig. 5**). It is critical to note that, by definition,
557 all of these MD clusters demonstrating a significant moderation effect overlapped with clusters
558 demonstrating an age effect and predicting fourth quartile frontal fast sleep spindle density (**Fig.**
559 **5**).

560 Therefore, white matter integrity, particularly within sensorimotor tracts including
561 posterior portions of the corpus callosum and posterior projection fibers containing connections
562 that were degraded in older adults, statistically influences whether or not sleep spindles promote
563 motor memory consolidation.

564

## 565 Discussion

566 Older adults have significantly impaired overnight motor memory consolidation relative
567 to young adults, and the reason for why this occurs has remained unknown. Here we identify that
568 white matter degeneration within specific sensorimotor tracts represents at least one mechanism
569 that accounts for both impaired frontal fast sleep spindle expression and sleep-dependent motor
570 memory consolidation. That is, the integrity of white matter structure in the adult human brain,
571 more than age per se, determines the presence or absence of sleep-spindle associated motor
572 memory consolidation.

573 In the current study, we demonstrate that the association between fast sleep spindles and
574 motor learning is strongest in the fourth quartile of stage 2 NREM sleep, and this effect is
575 diminished in older relative to young adults. While this time of night sensitivity is consistent
576 with previous work demonstrating a quartile effect of stage 2 NREM sleep on motor skill
577 learning (Walker et al., 2002), fast sleep spindles throughout stage 2 NREM sleep also

significantly predicted motor skill memory consolidation. It thus is unlikely that the last quartile is mechanistically unique with regards to sleep-dependent neuroplasticity.

However, the last quartile of the sleep period shows the highest sleep spindle activity and density, and also is when sleep spindle expression is impacted the most by age (Landolt et al., 1996; Carrier et al., 2011; Martin et al., 2013). Therefore, age-related reductions in sleep spindle-dependent motor skill memory consolidation may be especially sensitive to the effect of age at the time of night when sleep spindles typically predominate, i.e. the last quartile of stage 2 NREM sleep. The time of night effect may also relate to other NREM sleep oscillations. Slow waves causally support declarative memory consolidation (Marshall et al., 2006), predominate early in the night, and can often couple with sleep spindles during these phases. Sleep spindles later in the night appear to be less clustered with cortical slow waves, and with the lower incidence of cortical slow waves at this time of night, may be more able to support procedural motor skill memory consolidation. This is consistent with a recent causal manipulation using transcranial stimulation, demonstrating that targeted sleep spindle enhancement benefits motor skill memory most significantly during stage 2 NREM sleep, rather than during SWS (Lustenberger et al., 2016).

*White matter and sleep spindles*: Consistent with prior findings (De Gennaro and Ferrara, 2003; Martin et al., 2013; Mander et al., 2014), we identified reductions in fast sleep spindles in older relative to young adults that were topographically maximal over central and frontal EEG derivations. Furthermore, the extent of sleep spindle impairment, relative to young adults, increased as the night progressed, with the largest difference observed in the last quartile of the night. Extending prior work, we establish that one factor statistically predicting this now well-

600 replicated topographically and temporally specific spindle impairment is the degree of white
601 matter pathway deterioration in older adults.

602 In young adults, white matter in multiple fiber tracks explains inter-individual differences
603 in sleep spindle expression (Piantoni et al., 2013). Our findings show considerable overlap with
604 these previously reported fiber pathways, including the corpus callosum, suggesting that white
605 matter integrity within regions that account for individual differences in sleep spindle expression
606 in young adults also predicts age-related decline in fast sleep spindle expression. It should be
607 noted that the association between task-related fast sleep spindles and white matter that we
608 identified was topographically discreet, being unique to the fronto-central derivations. This is
609 relevant because it was these derivations that similarly show age-specific reductions in sleep
610 spindles in older relative to young adults, and were also regions that further predicted the success
611 of overnight motor memory consolidation in young adults.

612 This specificity supports the interpretation that sleep spindles that are expressed over
613 each EEG derivation may also be expressed within, or at least modulated significantly by,
614 regionally-specific properties of white matter fiber pathways that are associated with different
615 neurobehavioral functions and may exhibit age-related atrophy at distinct rates.

616 The current anatomical associations may further illuminate why the process of human
617 aging selectively reduces frontal sleep spindle expression while leaving posterior sleep spindle
618 expression relatively preserved (De Gennaro and Ferrara, 2003; Mander et al., 2014). Frontal
619 white matter fiber tracts deteriorate early and more severely with increasing age than posterior
620 and temporal white matter pathways (Salat et al., 2005). Based on the current findings, these
621 selective degenerative influences on white matter brain structure in older age appear to represent
622 one potentially important candidate mechanism explaining the regionally specific decline of the

623 fast sleep spindle oscillation across the lifespan. This interpretation is consistent with the known
624 anatomical basis of sleep spindles, derived from animal studies linking cortico-thalamic white
625 matter fiber integrity with sleep spindle expression (Steriade et al., 1987).

626 *White matter as a moderator of sleep-dependent memory*: Paralleling previous studies,
627 we demonstrated that older adults have significantly impaired overnight motor memory
628 consolidation relative to young adults. Prior studies have concluded from this evidence that
629 sleep-dependent motor memory mechanisms are absent or significantly diminished in older
630 adults (Spencer et al., 2007; Peters et al., 2008; Wilson et al., 2012; Fogel et al., 2013; Pace-
631 Schott and Spencer, 2013). However, our white matter brain structure findings help temper this
632 claim, and offer mechanistic insight into why this may be more true in some older individuals
633 than others.

634 Although white matter integrity was degraded in a large collection of fiber tracts in older
635 adults, relative to young adults, it was age-related degeneration in a select subset of these fiber
636 tracts that accounted for the topographic specificity and the magnitude of fast sleep spindle
637 reduction in older adults. Critically, the degeneration of the posterior corpus callosum in
638 particular, but also potentially the right tapetum and corona radiata white matter fibers,
639 significantly moderated the influence of these same topographically-specific fast sleep spindles
640 on motor skill memory consolidation in older adults.

641 The impairment in sleep spindle-dependent motor skill memory consolidation was
642 influenced by two non-mutually exclusive and co-occurring age-related impairments in sleep
643 spindles. First, age reduces sleep spindle expression, particularly within motor memory relevant
644 white matter tracts, and this reduced expression may be insufficient to support motor memory
645 consolidation. Second, age-related factors, including white matter degeneration, influences the

ability of sleep spindles that are expressed within these motor memory relevant tracts to facilitate the memory transformation necessary to promote motor skill memory consolidation.

It is unclear exactly how age-related degeneration of these specific white matter tracts contribute to disruptions in the transformation of motor skill memories supporting motor skill memory consolidation. The fiber paths demonstrating sleep- and age-related sensitivity in the current study are known to connect cortical regions over which sleep spindles are dominantly expressed. The deterioration of these pathways may therefore diminish the capacity of sleep spindles to influence the functional transformation of motor skill memories necessary to support procedural memory consolidation in the elderly. This is consistent with prior fMRI evidence demonstrating that aging alters the neural signature associated with sleep spindle-dependent offline motor skill memory transformation (Fogel et al., 2013). The relevance of these specific white matter tracts to sensorimotor function, and thus motor skill memory, is evident across five descriptive levels.

First, experimentally blocking myelination within the corpus callosum in rodents following novel motor learning abolishes long-term motor memory consolidation (McKenzie et al., 2014), indicating that properties of the corpus callosum are causally necessary to consolidate a newly acquired motor skill. Relatedly, increasing piano practice in human participants is associated with greater white matter integrity within the body and splenium of the corpus callosum (Bengtsson et al., 2005).

Second, cross-sectional and longitudinal studies in aging show that corpus callosum atrophy, particularly within the body and splenium of the corpus callosum, predicts deterioration of motor ability (Frederiksen et al., 2011; Ryberg et al., 2011; Koch et al., 2013).

668 Third, lesions within the corpus callosum can result in alien hand syndrome—a syndrome
669 where the control of complex goal-directed hand movements becomes aberrant, resulting in
670 impaired motor performance (Banks et al., 1989; Aboitiz et al., 2003). This is aligned with the
671 fact that the corpus callosum, as well as the right tapetum, is critical for integrating sensory
672 information to aid motor planning and selection (Tanaka et al., 1996; Lindner et al., 2010).

673 Fourth, projection fibers in the posterior corona radiata communicates parietal sensory
674 information that aids skilled motor actions, specifically those of the hand (Wakana et al., 2004;
675 Kim and Pope, 2005; Iguchi et al., 2006).

676 Fifth, functional neuroimaging studies consistently implicate the primary motor,
677 premotor, somatosensory cortex, parietal cortex, and basal ganglia in the overnight consolidation
678 of this motor skill task (Fischer et al., 2005; Walker et al., 2005; Fogel et al., 2013), relevant
679 considering that communication between all these regions depends on sufficient white matter
680 integrity within the body and splenium of the corpus callosum, the tapetum, and the posterior
681 corona radiata (Tanaka et al., 1996; Wakana et al., 2004; Huang et al., 2005; Kim and Pope,
682 2005; Iguchi et al., 2006; Lindner et al., 2010).

683 Beyond the relevance for explaining white matter anatomical associations with sleep
684 spindle oscillations, these same associations appear to have next-day functional-outcome
685 relevance, here in predicting the relative impairment in overnight motor skill memory
686 consolidation in older adults. Age-dependent deterioration in these white matter pathways may
687 therefore be one parsimonious factor explaining the discrepancy in the literature regarding why
688 some studies in older individuals exhibit impaired sleep-dependent motor memory (Spencer et
689 al., 2007; Fogel et al., 2013) while others show no evidence of overnight sleep-dependent
690 memory deficits whatsoever (Tucker et al., 2011). Notably, the cohort in the latter study in

691 which overnight improvement in MST performance was observed was composed of older adults
692 that were significantly younger than the older adults in the current study (difference = 5.5 years,
693 $P$=0.008). Building on our results, and based on the known association between age and white
694 matter fiber tract integrity, it is possible that greater white matter degeneration in our older
695 elderly cohort explains the lack of sleep-dependent motor skill memory consolidation reported.

696 Although these findings offer a first characterization of an age-related pathological
697 mechanisms accounting for the late-life failure of sleep spindle-related motor memory
698 consolidation, they raise several new questions. For example, it is now critical to know whether
699 the structural integrity of the aging brain determines the efficacy of pharmacological and/or non-
700 pharmacological sleep interventions for improving sleep in older adults, and as a consequence,
701 sleep-dependent cognition. In addition, it remains unknown whether structural features of brain
702 integrity predict the longitudinal decline in sleep physiology with the progression of age, and if
703 so, how this relationship interacts with cognitive decline. Finally, clarifying whether these sleep
704 physiology, brain structure, and memory associations are exaggerated in neurodegenerative
705 diseases already known to display grey and white matter anomalies, sleep disruption, and
706 cognitive impairment, and how these relationships are distinct between dissociable conditions
707 such as Alzheimer's disease, Parkinson's disease, and Fronto-Temporal dementia is of critical
708 importance (Petit et al., 2004; Terpening et al., 2013).

709

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718

## 719 Author contributions

720 B.A.M. designed the study, conducted the experiments, analyzed the data, and wrote the
721 manuscript. A.Z. and J.L. aided in data collection, analysis, and manuscript preparation. V.R.
722 aided in data analysis and manuscript preparation. B.L. aided in study screening procedures and
723 manuscript preparation, J.M.S. and S.V. provided data analytic tools, aided in data analysis, and
724 manuscript preparation. S.A.I aided in study design and manuscript preparation. W.J. provided
725 the elderly subject pool and data analytic tools, aided in study design and manuscript preparation.
726 M.P.W. designed the study, aided data analysis, and wrote the manuscript.

727

# Tables

Table 1. Demographic and Neuropsychological Measures (mean±s.d.)

| **Variable** | **Young (sleep; n=20)** | **Young (wake; n=20)** | **Older (sleep; n=31)** | **Older (wake; n=20)** |
|---|---|---|---|---|
| Age (yr) | 20.4±2.0 | 21.7±2.9 | 73.5±5.2 | 74.1±7.1 |
| Gender | 12 Female | 9 Female | 22 Female | 17 Female |
| MMSE | 29.6±0.9 | 29.4±1.0 | 29.5±1.0 | 29.4±0.9 |
| Mean prestudy bed time | 0:20±0:54 | 23:43±1:01 | 22:50±1:15 | 22:56±1:06 |
| Mean prestudy wake time | 8:26±0:52 | 7:34±0:53 | 7:16±1:11 | 6:41±1:09 |
| Mean prestudy time in bed (hr) | 8.11±0.58 | 8.01±0.67 | 8.42±0.73 | 7.83±0.84 |
| Mean prestudy sleep time (hr) | 7.75±0.61 | 7.52±0.85 | 7.14±1.00 | 7.28±1.00 |
| Mean prestudy sleep latency (min) | 15.7±9.2 | 22.5±26.4 | 35.7±42.2 | 17.6±19.1 |
| Mean prestudy sleep efficiency (%) | 95.6±3.4 | 93.8±6.8 | 86.0±11.5 | 92.9±6.7 |
| | | | | |
| *Neuropsychological Measures* | | | | |
| CVLT (long delay, # free recalled) | | | 11.0±3.1 | 12.9±2.8 |
| WMS (visual reproduction %) | | | 75.0±17.0 | 83.9±21.7 |
| Trailmaking B (seconds) | | | 72.2±34.5 | 80.0±33.3 |
| Stroop (# correct in 60 seconds) | | | 50.2±14.2 | 50.1±11.8 |

MMSE denotes mini mental state exam, CVLT denotes California Verbal Learning Test number recalled after 20 minute delay, WMS denotes Wechsler Memory Scale-Revised

* denotes $P<0.05$ FDR corrected (sleep versus wake groups)

735
736 Table 2. Sleep statistics (mean±s.e.m.)

| **Variable** | **Young (*n* = 20)** | **Older (*n* = 31)** |
|---|---|---|
| Total Recording Time (min) | 480.4±0.2 | 479.5±0.7 |
| Total Sleep Time (min) | 431.6±6.1 | 340.8±12.2*** |
| Sleep Latency (min) | 16.6±2.8 | 23.1±4.7 |
| Wake After Sleep Onset | 27.9±5.8 | 114.0±11.2*** |
| Stage 1 (min) | 14.3±1.6 | 22.4±1.5** |
| Stage 2 (min) | 201.1±6.7 | 192.1±10.4 |
| Slow Wave Sleep (min) | 117.9±7.1 | 61.7±6.5*** |
| Rapid Eye Movement Sleep (min) | 98.3±6.1 | 64.6±5.5*** |
| Sleep Efficiency (%) | 90.8±1.3 | 71.4±2.6*** |

737 *denotes *P<0.05, ** P<0.01, ***P<0.001*

738
739

740 Table 3. MD predicts sleep spindle density at CZ

| JHU Atlas Label | JHU # | $P_{FDR}$ |
|---|---|---|
| corpus callosum genu | 3 | 0.021* |
| corpus callosum body | 4 | 0.005* |
| corpus callosum splenium | 5 | 0.003* |
| R ant. limb of the internal capsule | 17 | 0.008* |
| R post. limb of the internal capsule | 19 | 0.005 |
| R retrolenticular limb of the internal capsule | 21 | 0.005* |
| L retrolenticular limb of the internal capsule | 22 | 0.003 |
| R ant. corona radiata | 23 | 0.011* |
| R sup. corona radiata | 25 | 0.009* |
| R post. corona radiata | 27 | 0.005* |
| L post. corona radiata | 28 | 0.008* |
| R post. thalamic radiation | 29 | 0.003* |
| L post. thalamic radiation | 30 | 0.003* |
| R sagittal stratum | 31 | 0.003* |
| R external capsule | 33 | 0.003* |
| R fornix/stria terminalis | 39 | 0.005* |
| L fornix/stria terminalis | 40 | 0.005* |
| R sup. longitudinal fasciculus | 41 | 0.011* |
| R sup. fronto-occipital fasciculus | 43 | 0.008* |
| R tapetum | 47 | 0.013* |

741 * denotes a significant age effect in MD was also detected at $P<0.05$ FDR corrected,
742 MD denotes white matter mean diffusivity, R denotes right hemisphere, L denotes left hemisphere,
743 ant. denotes anterior, post. denotes posterior, sup. denotes superior
744 Presented effects are adjusted for age group

## Figure Legends

**Figure 1.** Behavioral performance on the motor skill task (MST) in young (red) and older (light blue) adults who slept, and young (orange) and older (green) adults who remained awake between training and retest. (**a**) Performance speed (number of correct sequences typed per 30 s trial; top panel) and accuracy (error rate; bottom panel) during training (trials 1-12) and 10-12 hour retest (trials 13-16). Baseline (trials 1-2), post-training (trials 11-12), and retest (trials 14-16) trials used in analysis are outlined by the dashed box. (**b**) Percent change in speed (top panel) and accuracy (bottom panel) from post-training (trial 11-12 mean) to 10-12 hour retest (trial 14-16 mean). Inclusion of measures of the change in reaction time across training (between sequence mean reaction time, within sequence mean reaction time, standard deviation of reaction time) in an ANOVA model examining the change in MST speed from post-training to retest does not appreciably change the results. Significant trial by age group and trial by sleep condition group interactions still remain (all $P<0.01$), despite no significant differences in MST speed during training. Values are represented as mean ± s.e.m.

*denotes *P<0.05, ** P<0.01, ***P<0.001 on within and between subjects post hoc tests*

**Figure 2.** (**a**) Two dimensional topoplots of the impact of age on stage 2 NREM fast sleep spindle density (13.5-15Hz) across the entire night (left most topoplot) and during all four quartiles of stage 2 NREM sleep. Fast sleep spindle density during stage 2 NREM sleep for young (red) and older (light blue) adults at CZ, the derivation exhibiting the greatest difference in spindle density across age groups, during each quartile of stage 2 NREM sleep is plotted in the bottom left panel. (**b**) Two dimensional topoplot of the fitted slope of the change in effect size of the age difference in stage 2 NREM fast sleep spindle density across quartiles. Warmer colors reflect positive increases in effect size across the night, i.e. the difference between young and older adults in stage 2 NREM fast sleep spindle density progressively increases across the night. The effect size of the difference in fast sleep spindle density during stage 2 NREM sleep between young and older adults at FZ (dark orange), CZ (light orange), and PZ (yellow) is plotted for each quartile in the bottom right panel.

*(white) denotes $P<0.05$, *(red) denotes $P<0.05$ FDR corrected across 76 comparisons

**Figure 3.** (**a**) Two dimensional topoplot of the association between stage 2 NREM fast sleep spindle density and percent overnight change in MST performance. (**b**) A scatter plot of this association with mean stage 2 NREM fast sleep spindle density over the entire sleep period at CZ is presented below (bottom left) for young (red) and older (light blue) adults. (**c**) Two dimensional topoplot of the association between fourth quartile stage 2 NREM fast sleep spindle density and percent overnight change in MST performance. (**d**) A scatter plot of this association with mean stage 2 NREM sleep spindle density during the fourth quartile at CZ is presented below (bottom right) for young (red) and older (light blue) adults.

* denotes $P<0.05$ uncorrected (white) and FDR corrected (light blue)

783 **Figure 4.** Associations between white matter mean diffusivity, age group, fourth quartile stage 2
784 NREM fast sleep spindle density at CZ, and white matter lesion (WML) volume. (**a**) Voxelwise
785 comparison of the increase in white matter mean diffusivity (MD) in older relative to young
786 adults (in grayscale). (**b**) Fourth quartile stage 2 NREM fast sleep spindle density at CZ
787 predicting white matter MD while controlling for age group (in hot colors), overlaid on clusters
788 demonstrating age effects (in grayscale). Effects are presented at $P<0.05$ family-wise error
789 (FWE) whole brain corrected at the cluster level. The mean extracted white matter MD across all
790 significant voxels associated with fourth quartile stage 2 NREM fast sleep spindle density at Cz
791 in young (red) and older (light blue) adults is presented below. (**c**) Scatter plot of the association
792 between fourth quartile stage 2 NREM fast sleep spindle density at CZ and mean extracted MD
793 in young (red) and older (light blue) adults. (**d**) Scatter plot of the association between white
794 matter lesion (WML) volume and the mean extracted white matter MD across all significant
795 voxels associated with fourth quartile stage 2 NREM fast sleep spindle density at CZ in older
796 adults.

797 *** denotes $P<0.001$

798 **Figure 5.** White matter integrity moderates the association between fourth quartile stage 2
799 NREM fast sleep spindle density at CZ and motor memory consolidation. (**a**) Age effect on
800 white matter MD are presented in grayscale. Fourth quartile stage 2 NREM fast sleep spindle
801 density at CZ predicting white matter mean diffusivity (MD) while controlling for age group is
802 presented in hot colors. Voxels in this cluster that significantly moderate the relationship
803 between fourth quartile stage 2 NREM fast sleep spindle density at CZ and motor memory
804 consolidation while controlling for age group are presented in cool colors. (**b**) An interaction plot
805 demonstrating the influence of the mean white matter MD across the body and splenium of the
806 corpus callosum, the right posterior corona radiata, and the right tapetum on the association
807 between fourth quartile stage 2 NREM fast sleep spindle density at CZ and motor memory
808 consolidation. Original data are plotted with color outlining young (red) and older (light blue)
809 adults for reference, with regression lines shown for participants with white matter MD values
810 (denoted WM MD) less than the mean (black line) and ≥ the mean (black dashed line) across all
811 participants. This plot reveals that as white matter integrity decreases, sleep spindles offer less of
812 a benefit to motor memory consolidation even while controlling for age.
813

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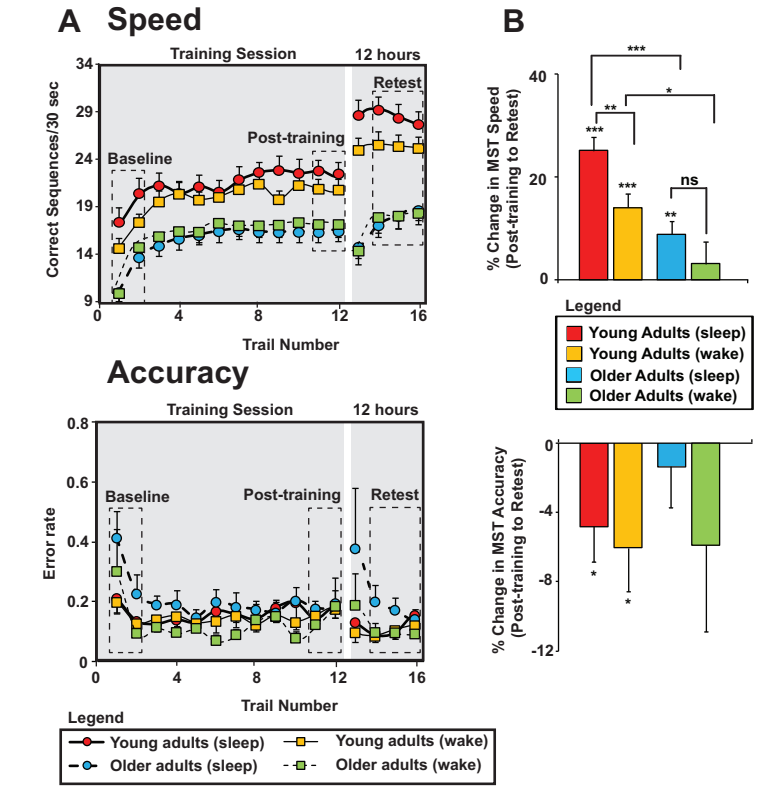

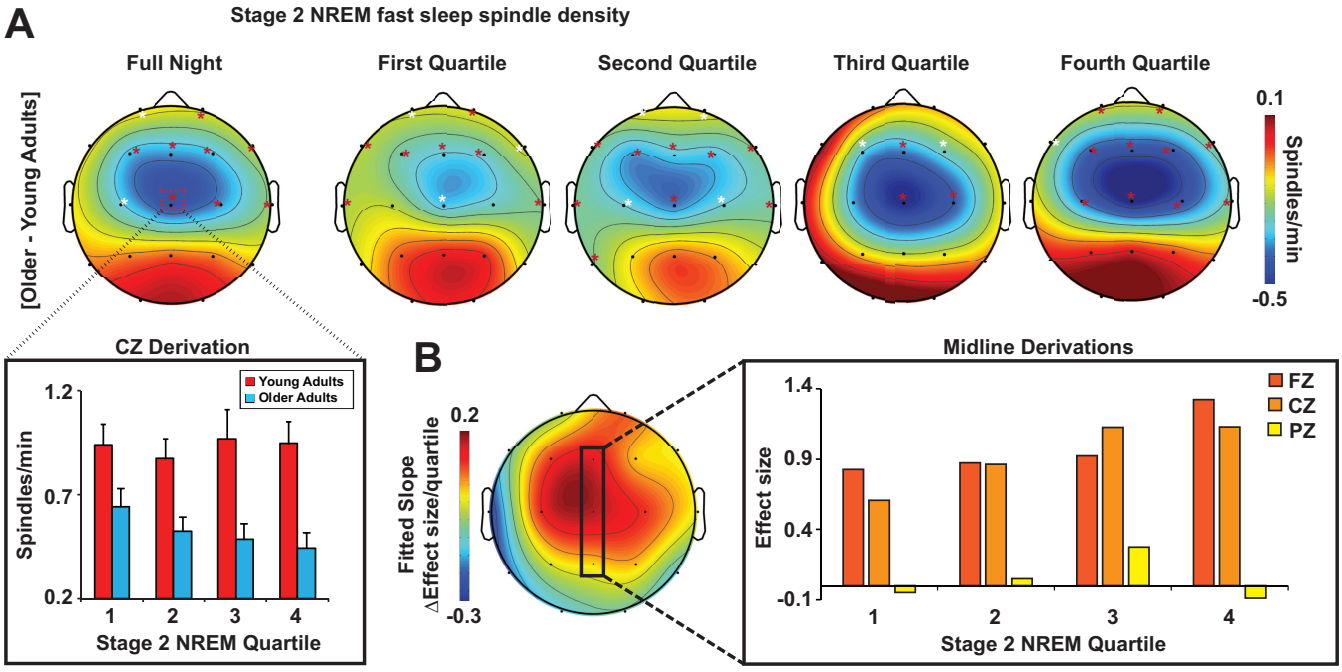

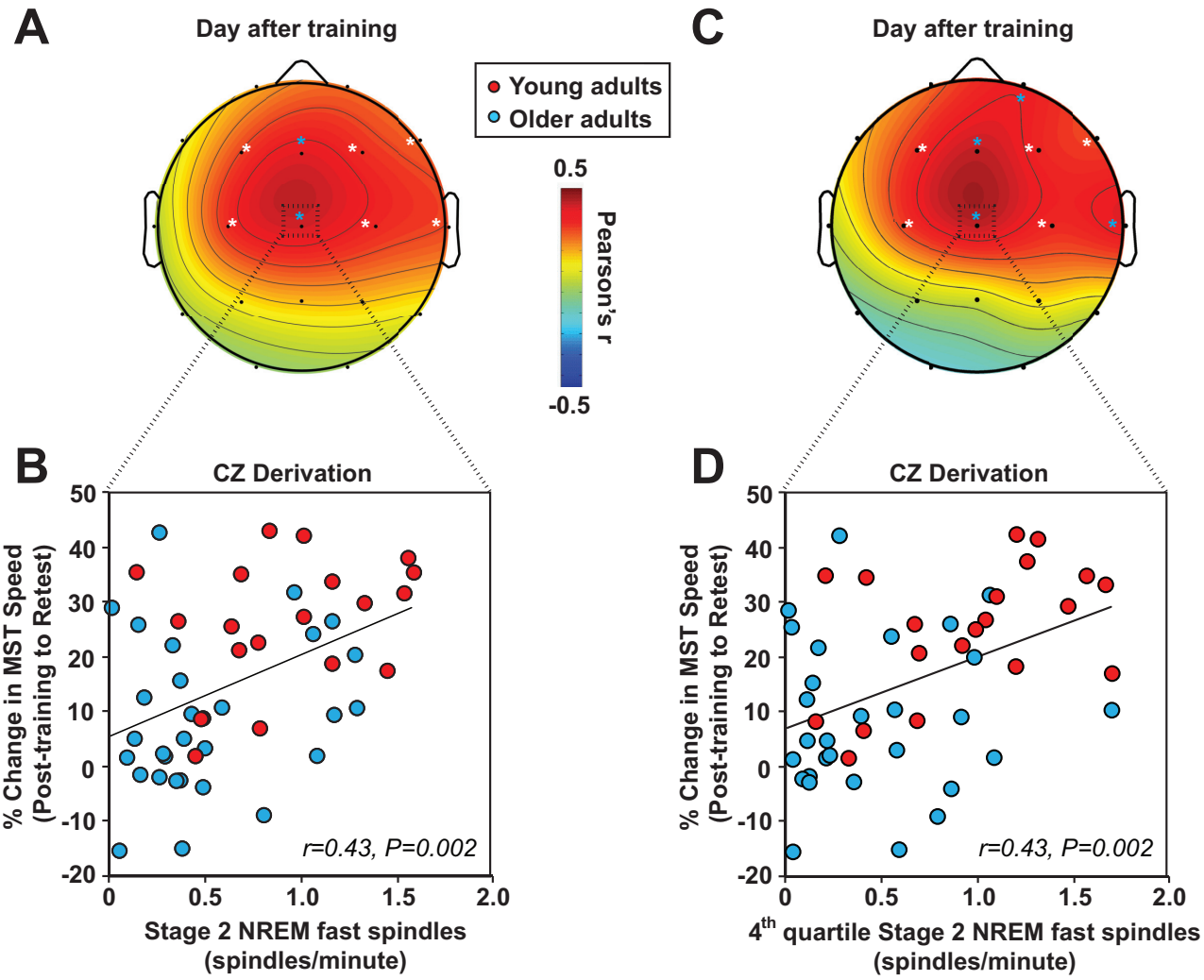

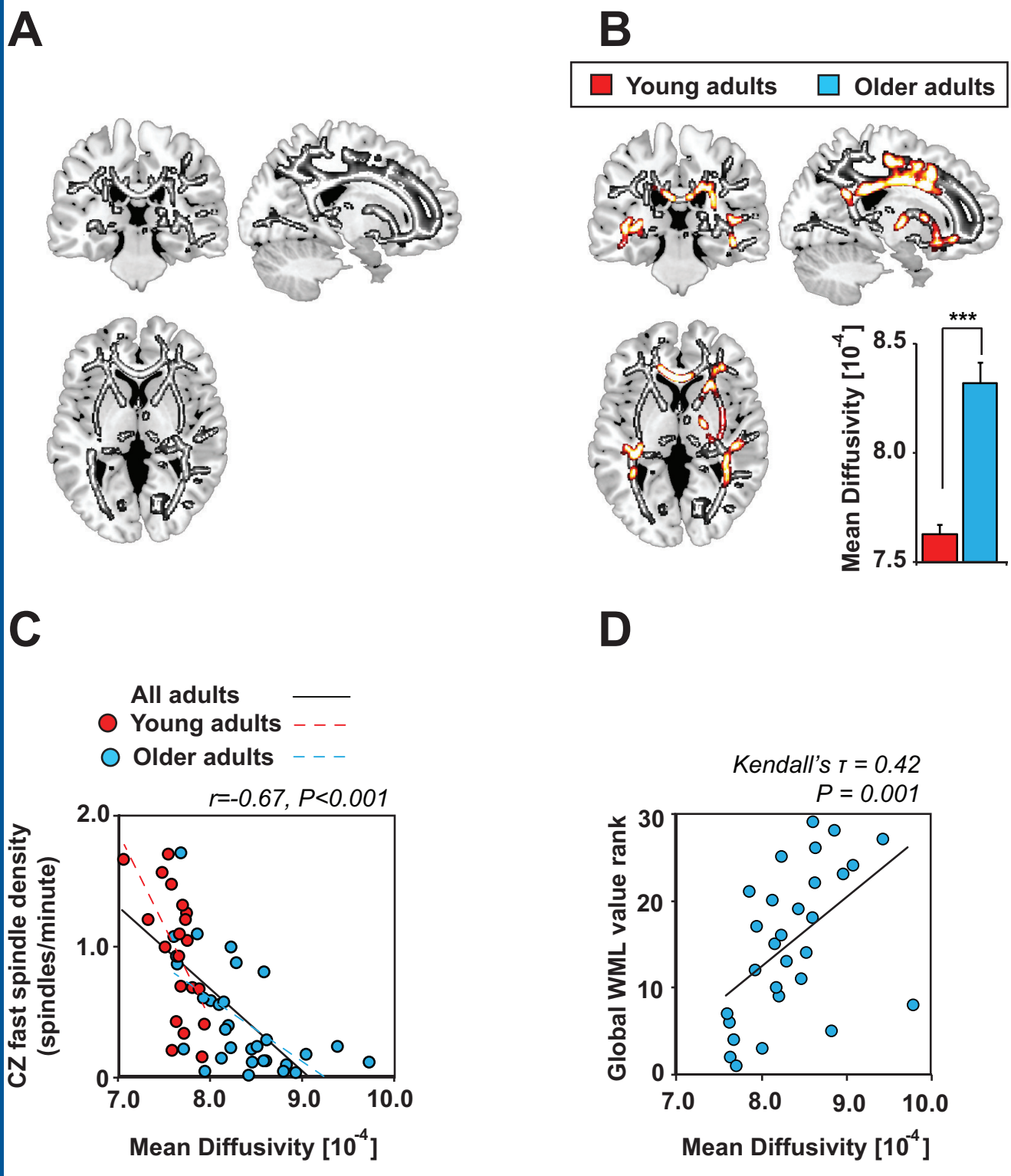

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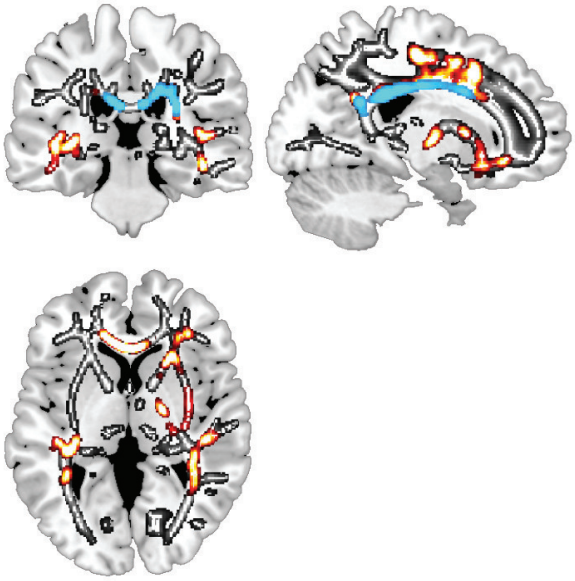

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