The Role of Sleep in Brain Health in Normal Aging and in Alzheimer's Disease

By

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ABSTRACT

Alzheimer's disease (AD) is an immense and growing health, social and economic burden, without cure. Therapies are needed urgently to delay AD or reduce its severity. The hallmark pathologies of AD are accumulation of amyloid and tau in the brain, which begins to develop decades before dementia emerges. As pathology accumulates, symptoms progress from an asymptomatic preclinical phase, to Mild Cognitive Impairment (MCI) and finally dementia. Disease-modifying drug trials in AD dementia patients have been unsuccessful, suggesting that earlier intervention may be necessary, to slow AD pathology and thereby delay or diminish clinical symptoms. Sleep is a promising source of early disease markers and targets for early intervention, because converging evidence links sleep with early AD pathogenesis.

The aim of this dissertation work was to determine the extent to which sleep disturbance is associated with preclinical AD pathology. I measured sleep and biomarkers of AD pathology in cognitively healthy, middle aged adults at risk for AD. I found that self-report of poor sleep was associated with cortical amyloid burden (imaged with positron emission tomography and measured in cerebrospinal fluid; CSF), and CSF markers of axonal degeneration, neurofibrillary tangles and neuro-inflammation. Using high density electroencephalography (hdEEG), I showed that AD pathology is also associated with changes in sleeping brain activity in the slow wave range, which is critical for memory and the restorative function of sleep. I characterized changes in EEG topography across healthy aging, to show that the AD-related alterations follow a distinct pattern from normal aging.

Based on these findings, sleep health may be a modifiable risk factor for AD, for which many effective treatments are already available. Furthermore, sleep EEG may be a powerful tool for detecting very early biomarkers of AD neuropathology. Further studies are needed to identify the aspects of sleep that are most amenable to modification, the sleep interventions that most

effectively impact AD pathology and symptoms, and to understand the mechanistic pathways through which sleep and AD pathogenic processes interact, with the ultimately goal of delaying AD or diminish AD symptoms.

CHAPTER 1

Introduction

INTRODUCTION

In 2014 approximately 5.2 million Americans had Alzheimer's disease (AD), which cost $214 billion in health care spending, and affected the health and finances of 15.5 million caregivers. Prevalence is projected to triple by 2050 as the population ages (Alzheimer's Association 2014). No cure exists and trials of disease-modifying treatments targeting late stage disease have been unsuccessful.(Schneider *et al.* 2014b) Earlier intervention may be necessary, before irreversible neural damage has occurred, therefore identification of treatment targets and disease markers in earlier disease is essential (Sperling *et al.* 2011; Selkoe 2012). Converging evidence suggests a promising role for sleep in detection and/or intervention. However, it remains unclear which aspects of sleep are related to early AD pathology. Therefore, the objective of this dissertation work is to identify sleep characteristics associated with AD pathology and risk in midlife, when intervention may be most effective.

Preclinical Alzheimer's Disease

AD is a progressive neurodegenerative disease, and AD brain pathology develops as early as decades before dementia emerges.(Jack *et al.* 2013) As pathology accumulates, symptoms progress from an asymptomatic preclinical phase, to Mild Cognitive Impairment (MCI) and finally dementia (Sperling *et al.* 2011). Studying the preclinical phase presents a challenge, since there are no definitive diagnostic markers at this stage. One approach is to study individuals who are currently cognitively healthy, but carry risk factors for AD. Two cohorts of such individuals were employed in this dissertation research: participants of the Wisconsin Alzheimer's Disease Research Center (WADRC) and the Wisconsin Registry for Alzheimer's Prevention (WRAP). Both cohorts are designed to identify biological and lifestyle factors that may increase AD risk, and have higher rates than the general population of the two potent risk factors for sporadic AD: a parental family history of AD and carriage of the ε4 allele of the APOE gene (Sprecher *et al.* In Press; Sager, Hermann and La Rue 2005; Koscik *et al.* 2016). Participants are enrolled in

middle age while cognitively healthy, and are followed longitudinally with neurocognitive and health assessments. Many also elect to undergo additional procedures including neuroimaging, collection of cerebrospinal fluid, and sleep assessments. Children of AD patients are 6 times more likely to develop AD (Mayeux *et al.* 1991; Green *et al.* 2002; Jayadev *et al.* 2008) and a family history of AD is associated with preclinical brain change (Bassett *et al.* 2006; Johnson *et al.* 2006, 2007; Trivedi *et al.* 2008; Fleisher *et al.* 2009; Xu *et al.* 2009; Bendlin *et al.* 2010b; Xiong *et al.* 2011; Okonkwo *et al.* 2012a, 2012b; Johnson *et al.* 2013). Carriage of the ε4 allele of the APOE gene is the main genetic risk factor for AD (Corder *et al.* 1993; Farrer *et al.* 1997).

Preclinical Alzheimer's Disease Pathology and Biomarkers

The two pathologic features that define AD are amyloid plaques and neurofibrillary tangles (McKhann *et al.* 2011). Both begin to develop years before cognitive deficits manifest, during the preclinical phase of AD (Jack *et al.* 2013). The pathogenic process of AD is still the subject of intensive research and debate. One leading hypothesis is the amyloid cascade, which posits that through overproduction or reduced clearance, soluble amyloid beta-42 (Aβ42) accumulates in the brain's extracellular space, then aggregates to form oligomers that ultimately accumulate in insoluble extracellular amyloid plaques (Sperling *et al.* 2011). Amyloid plaques can be detected in vivo using positron emission tomography (PET), and the most widely used amyloid-specific PET tracer is Pittsburgh Compound B (PiB) (Klunk *et al.* 2004). Another biomarker of greater amyloid plaque burden is decreased levels of Aβ42 in cerebrospinal fluid (CSF).

Tau is a structural component of axonal microtubules, which in AD becomes hyperphosphorylated and aggregates extracellularly in neurofibrillary tangles. In AD, CSF levels of total tau (T-tau) increase over time, which is thought to reflect tau's passage out of the neuron due to continued degeneration of axons (Zetterberg 2017). CSF levels of phosphorylated tau (P-tau) also increase progressively though the course of AD, and correlate with neurofibrillary

tangles (Buerger *et al.* 2006; Seppälä *et al.* 2012). PET tracers specific to tau are currently still under development (Saint-Aubert *et al.* 2017).

Sleep and Alzheimer's Disease

Epidemiologic studies show that abnormal sleep-wake patterns and sleep disordered breathing are associated with increased risk of dementia (Tranah *et al.* 2011; Yaffe *et al.* 2011; Yaffe, Nettiksimmons and Byers 2014). One pathway through which this risk may be conferred is sleep's modulation of amyloid metabolism. Aβ42 levels in CSF normally follow a diurnal rhythm (Kang *et al.* 2009; Roh *et al.* 2012) and a single night of sleep deprivation dramatically attenuated the morning nadir of CSF Aβ42 in healthy middle-aged men (Ooms *et al.* 2014). Sleep promotes Aβ clearance via the glymphatic system (Xie *et al.* 2013), therefore disturbed sleep could promote amyloid accumulation. Indeed, older adults with pathological levels of CSF Aβ42 had more fragmented sleep (Ju *et al.* 2013). Obstructive sleep apnea (OSA) is a common disorder in which the airway closes repeatedly during sleep, producing sleep fragmentation and hypoxemia. Both hypoxia and sleep fragmentation increased amyloid deposition in rodents (Kang *et al.* 2009; Shiota *et al.* 2013a), and in elderly humans OSA severity correlated with CSF Aβ42 (Osorio *et al.* 2014). Tau pathology is also associated with sleep deficits. A mouse model expressing a human mutation, slept less and had fewer transitions between wake and sleep, suggesting impaired regulation of state transitions (Holth *et al.* 2017).

25-30% of US adults have a sleep disorder (National Center on Sleep Disorders Research 2011) and many effective FDA-approved methods already exist to improve sleep. The findings described above suggest that sleep disruption may be a modifiable risk factor for AD, for which treatments are already available. Treatment of sleep disordered breathing and irregular sleep-wake patterns in AD patients deepens sleep and improves cognition (Ancoli-Israel *et al.* 2008; Cooke *et al.* 2009a). However, these sleep treatments do not reverse dementia. Therefore the

work in this dissertation sought to identify sleep factors that could be intervention targets in preclinical AD, to prevent or delay dementia due to AD.

Alzheimer's Disease Biomarkers

As described above, sleep restriction promotes amyloid deposition in rodent models of AD (Kang *et al.* 2009). Conversely, other experiments have shown that sleep becomes increasingly disturbed as amyloid burden increases, and is normalized by amyloid clearance (Roh *et al.* 2012). These data suggest a bidirectional relationship between sleep and amyloid, such that sleep disturbance may be a cause and/or a symptom of amyloid deposition. To the extent that sleep is a symptom of AD neuropathology, sleep may harbor biomarkers of early AD.

Current AD biomarkers can be invasive (e.g. lumbar puncture for CSF), expensive (e.g. PET and MRI), non-portable, or have significant patient exclusions (e.g. MRI ineligibility due to metal in body). By contrast sleep monitoring is non-invasive, inexpensive, portable and well-tolerated. Electroencephalography (EEG) during sleep may detect very early changes in brain health. EEG arises from synchronous firing of pyramidal neurons, and is sensitive to both structure and dynamic function of the brain (Steriade 2003; Piantoni *et al.* 2013). Amyloid plaques impair the ability of cortical neurons to fire synchronously (Stern *et al.* 2004), and synaptic loss (Terry *et al.* 1991) and neuronal dysfunction (Okonkwo *et al.* 2014) are early features of AD that are highly correlated with cognitive decline. Slow waves and spindles are sleep EEG waveforms important for sleep-dependent memory processes (Pace-Schott and Spencer 2014). Patients with AD or Mild Cognitive Impairment (a prodrome of AD) show reductions in slow waves and spindles that correlate with memory deficits (Rauchs *et al.* 2008; Westerberg *et al.* 2012).

Sleeping brain activity is not a global cortical phenomenon, but can be regionally heterogeneous. Sleep EEG has a characteristic spatial distribution across the scalp, which develops from infancy through to adulthood (Kurth *et al.* 2010; Piantoni *et al.* 2013) and is stable

from night to night within an individual (Finelli, Achermann and Borbély 2001; De Gennaro *et al.* 2005). AD patients can be distinguished from MCI by differences in tempero-parietal *waking* EEG power (Hatz *et al.* 2013), but preclinical AD had not been tested at the time of this dissertation. Sleep EEG is more sensitive to subtle changes in network activity than waking EEG as it is not confounded by fluctuating attention or the cognitive capacity to follow instructions. Thus EEG with high spatial resolution (high density EEG; hdEEG) during sleep may be sensitive to very early AD-related changes in brain function, before damage has reached the detection threshold of other imaging methods. Sleep-based biomarkers could be useful for detection of preclinical AD, enrichment of clinical trial samples, and to generate testable hypotheses about disease mechanisms.

OVERVIEW AND SUMMARY OF SPECIFIC AIMS

Together, these findings suggest that sleep may harbor both treatment targets and early biomarkers of AD. However, it remains unclear which aspects of sleep are related to early AD pathology. The objective of this dissertation is to identify sleep characteristics associated with AD pathology and risk in midlife, when intervention may be most effective. My central hypotheses are that sleep disturbance promotes AD pathology, and that preclinical AD brain change is detectable by sleep hdEEG. The rationale for this work is that identifying sleep factors associated with preclinical AD is an essential starting point for developing sleep-based treatments and/or sleep-based biomarkers for preclinical AD detection. To address these hypotheses I proposed 2 specific aims:

Aim 1: Identify sleep characteristics that are linked with midlife AD neuropathology.

Methods of early detection and intervention are critically needed for AD. Converging evidence suggests a link between sleep and AD pathology but it is unclear which aspects of sleep would be most useful as treatment targets or potential biomarkers. The objective of this aim is to

determine which aspects of sleep are associated with early AD pathology in humans, with the working hypothesis that poorer sleep is associated with greater AD pathology in cognitively intact late-middle-aged adults.

Aim 1.1: Determine the extent to which sleep is linked with PET biomarkers of midlife beta amyloid. I hypothesized that poor sleep would be associated with greater amyloid deposition. To address this aim I tested the association of brain amyloid burden (measured with PET) with self-reported sleep quality, in cognitively normal adults in late middle age. This work is described in Chapter 2.

Aim 1.2: Determine the extent to which sleep is linked with CSF biomarkers of midlife AD neuropathology.

I hypothesized that poor sleep would be associated with elevated CSF biomarkers of AD-related neuropathology. To address this aim I tested the association of self-reported sleep quality with CSF biomarkers of amyloid mis-processing and plaques (Aβ42), tau pathology (P-tau181), neuronal/axonal degeneration (T-tau, NFL), neuroinflammation/astroglial activation (MCP-1, YKL-40) and synaptic dysfunction/degeneration (neurogranin), in cognitively normal adults in late-middle age. This work is described in Chapter 3.

Aim 2: Determine the extent to which sleep EEG is altered in preclincal AD.

Currently there are few strategies for detecting AD early in the disease process. Sleep hdEEG is a promising tool because it is sensitive to subtle brain change, and is relatively inexpensive, non-invasive and portable. The objective of this aim is to determine whether preclinical AD is associated with altered sleep hdEEG topography.

AIM 2.1: Characterize changes in sleep EEG topography across normal aging.

Previous reports of sleep EEG changes in healthy aging had very low spatial resolution, and compared groups of young to older people, rather than across the age spectrum. Therefore I characterized age-related changes in sleep EEG in healthy adults aged 18-65 years, using hdEEG, which has high spatial resolution to detect regional changes in brain activity. This work provides a normative baseline with which to compare investigations of sleep EEG topography in preclinical AD, and is described in Chapter 4.

AIM 2.2: Determine whether altered sleep EEG topography is associated with midlife AD neuropathology.

I conducted hdEEG sleep recordings to investigate the relationship of regional sleeping brain activity with CSF biomarkers of amyloid and tau pathology. I hypothesized that preclinical AD pathology would be associated with lower regional EEG power in the slow wave range (1-4.5Hz, SWA). This work is described in Chapter 5.

CHAPTER 2

Amyloid Burden Is Associated With Self-Reported Sleep In Non-Demented Late Middle-Aged Adults

Amyloid Burden Is Associated With Self-Reported Sleep In Non-Demented Late Middle-Aged Adults

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ABSTRACT

Midlife may be an ideal window for intervention in Alzheimer's disease (AD). To determine whether sleep is associated with early signs of AD neuropathology (amyloid deposition) in late midlife, we imaged brain amyloid deposits using Positron Emission Tomography (PET) with [C-11]Pittsburgh Compound B (PiB), and assessed sleep with the Epworth Sleepiness Scale (ESS) and the Medical Outcomes Study (MOS) Sleep Scale in 98 cognitively healthy adults (aged 62.4 ± 5.7 years) from the Wisconsin Registry for Alzheimer's Prevention. We used multiple regression to test the extent to which sleep scores predicted regional amyloid burden. Participants reporting less adequate sleep, more sleep problems and greater somnolence on the MOS had greater amyloid burden in AD-sensitive brain regions (angular gyrus, frontal medial orbital cortex, cingulate gyrus and precuneus). Amyloid was not associated with reported sleep amount, symptoms of sleep disordered breathing, trouble falling asleep or ESS. Poor sleep may be a risk factor for AD and a potential early marker of AD or target for preventative interventions in mid-life.

INTRODUCTION

Amyloid plaques are a hallmark of Alzheimer's disease (AD). Accumulation and aggregation of the peptide β-amyloid (1-42) (Aβ42) into insoluble plaques is evident a decade or more before AD symptoms appear, during the preclinical phase of the disease (Sperling *et al.* 2011; Jack *et al.* 2013) and is thought to be a major cause of neural dysfunction and cognitive decline to dementia. Older adults (mean age 65.6 years) with pathological levels of Aβ42 in cerebrospinal fluid (CSF) had lower sleep efficiency as measured by actigraphy than those with normal Aβ42 levels (Ju *et al.* 2013). In humans, amyloid plaques can be imaged with positron emission tomography (PET) using radioligands such as [C-11]Pittsburgh Compound B (PiB). In older adults (mean age 78.2 years), greater amyloid burden was associated with self-report of poor sleep quality and shorter sleep duration (Spira *et al.* 2013).

The mechanism linking poor sleep with greater amyloid burden is not clear. In mice, sleep disruption increases amyloid generation (Shiota *et al.* 2013b) and deposition (Kang *et al.* 2009). Amyloid levels in brain interstitial fluid follow a diurnal pattern (Kang *et al.* 2009; Roh *et al.* 2012) and clearance of exogenous amyloid is greatest during sleep (Xie *et al.* 2013). Aβ plaques arise from an imbalance between Aβ production and clearance (Yan *et al.* 2009). Thus sleep problems may reduce Aβ clearance, leading to its accumulation and aggregation into plaques.

The association between sleep and amyloid burden has not been examined in late middle age. This age range is important because amyloid accumulation begins years before AD symptoms begin, and current AD treatments targeting later-stage disease have shown disappointing results (Schneider *et al.* 2014b). Earlier intervention may be a more effective strategy to prevent or delay clinical symptom onset due to AD pathology (Sperling *et al.* 2011). Sleep is an attractive therapeutic target because well-established methods already exist for improving sleep. Alternatively, if sleep is affected by amyloid deposition, sleep may harbor markers of early, preclinical AD useful for prognosis and treatment monitoring.

The objective of this study was to determine whether sleep quality and quantity are related to amyloid burden in late mid-life, and to determine which aspects of sleep are associated with increased amyloid burden. We used PiB PET imaging and validated sleep questionnaires to test the hypothesis that in cognitively healthy middle-aged adults, poorer self-reported sleep quality would be associated with greater amyloid burden in brain regions typically affected by AD.

METHODS

Participants and Study Design

Participants were drawn from the Wisconsin Registry for Alzheimer's Prevention (WRAP), a longitudinal cohort of >1500 cognitively healthy adults, aged 40-65 years at study entry (Sager, Hermann and La Rue 2005). Participants were included in the present analysis if they had completed the WRAP Wave 4 visit, which included sleep assessment, and had completed a PiB PET scan; 98 individuals met inclusion criteria. Pertinent demographic and cognitive characteristics are summarized in Table 2; note that the sample was enriched with parental family history of AD and APOE4 genotype, to a similar degree as the entire WRAP cohort.

WRAP participants underwent comprehensive neurocognitive and medical history assessment at baseline, four years later, and every 2 years thereafter at the University of Wisconsin (Sager, Hermann and La Rue 2005). Participants were recruited to PiB PET imaging sub-studies by telephone, letter, or in person at their WRAP visit. The scan closest to the time of the sleep questionnaires was used in this analysis. Exclusion criteria included MRI contra-indications, abnormal structural MRI, and diagnosis of significant neurological disease, medical illness or major psychiatric disorders (determined by patient self report). All study procedures were approved by the University of Wisconsin Institutional Review Board and each participant provided signed informed consent before participation.

MRI Acquisition and Processing

All participants were scanned on a GE 3.0 Tesla MR750 (Waukesha, WI) using an 8 channel head coil. A T1-weighted brain volume was acquired in the axial plane with a 3D inversion recovery prepared fast spoiled gradient-echo (3D SPGR) sequence using the following parameters: TI = 450 ms; TR = 8.1 ms; TE = 3.2 ms; flip angle = 12°; acquisition matrix = 256 × 256 ×156 mm, FOV = 256 mm; slice thickness = 1.0 mm. Voxels were 1 mm isotropic. The image acquisition protocol also included T2 weighted and FLAIR anatomical scans, which were reviewed by a neuroradiologist (H.A.R.) for exclusionary abnormalities. The T1-weighted volume was segmented into tissue classes using the updated segmentation feature in SPM12 (www.fil.ion.ucl.ac.uk/spm).

PiB PET Imaging

[C-11] PiB PET radiochemical synthesis, acquisition parameters and generation of distribution volume ratio (DVR) maps were detailed previously (Johnson *et al.* 2014). Briefly, after a 70 minute dynamic [C-11]PiB PET acquisition, PET data were reconstructed using a filtered back-projection algorithm (DIFT) and were corrected for random events, attenuation of annihilation radiation, deadtime, scanner normalization, and scatter radiation and were realigned and coregistered in SPM12. The data were then transformed into voxel-wise DVR maps of [C-11]PiB binding using the time activity curve in the gray matter (GM) of the cerebellum as the reference region (Logan *et al.* 1996).

Cortical Amyloid Burden Quantification

To reduce the number of statistical tests, amyloid binding was averaged within eight bilateral regions of interest (ROIs), selected on the basis of AD sensitivity and known amyloid binding. The eight ROIs from the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer *et al.* 2002) were angular gyrus, anterior cingulate gyrus, posterior cingulate gyrus, frontal medial orbital gyrus, precuneus, supramarginal gyrus, middle temporal gyrus, and superior temporal

gyrus (Figure 1). The inverse deformation field resulting from unified segmentation on each subject image was applied to each AAL ROI to produce ROI masks in native space. To constrain ROI analyses to grey matter only, each ROI mask was next multiplied by the binarized grey matter probability map thresholded at 0.3. A summary measure of amyloid burden was calculated by averaging all ROI means.

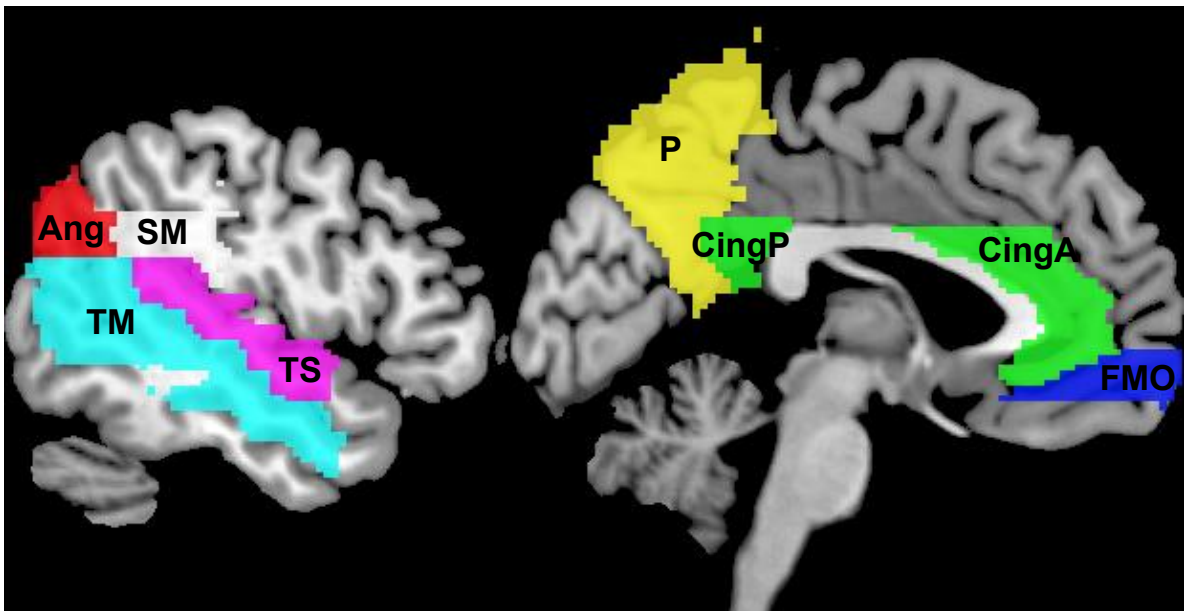

Figure 1. AAL Regions of Interest. Ang, angular gyrus; CingA, anterior cingulate; CingP, posterior cingulate; FMO, frontal middle orbital gyrus; P, precuneus; SM, supramarginal gyrus; TM, middle temporal gyrus; TS, superior temporal gyrus.

Sleep Assessment

Two validated questionnaires assessing sleep were completed as part of a larger standardized neuropsychological assessment, proximal to the time of the PET scan. The Epworth Sleepiness Scale (ESS) (Johns 1991) assesses sleep propensity and daytime sleepiness. Participants rate how likely they are to doze off or fall asleep in 8 common situations that vary in their soporific qualities, such as watching TV, talking to someone or lying down. Responses are on a 4-point scale ranging from 0 = “would never doze” to 3 = “high chance of dozing”. Responses are summed to produce a total score ranging from 0 to 24, with higher scores indicating greater

daytime sleepiness. The ESS has been shown to have good internal consistency (Cronbach α = 0.73-0.88) and test-retest reliability (correlation of measures across a 5-month interval = 0.82) (Johns 1992).

The Sleep Scale from the Medical Outcomes Study (MOS) (Hays and Stewart 1992) is summarized in Table 1. It comprises 12 questions about the past 4 weeks, from which 8 scores were computed. The first question asks how long it takes to fall asleep, with possible responses in 15 minute increments ranging from 1 = "0-15 minutes' to 5 = "More than 60 minutes". The second question asks the average number of hours slept each night, which is entered freely. Responses to the remaining 10 questions are on a 6-point scale ranging from 1="All of the time" to 6="None of the Time". Responses were converted to a 0-100 scale, with higher values indicating more of the concept being measured, then summed to give scores for 6 sleep domains: Sleep Disturbance, Somnolence, Sleep Adequacy, Snoring, Awaking Short of Breath or with a headache and Sleep Quantity, and two indices of sleep problems summarizing 6 (Index I) or 9 (Index II) items (Spritzer and Hays 2003). Multi-item scores show good internal consistency (Cronbach's alpha 0.71 to 0.81) (Viala-Danten *et al.* 2008). Table 1 indicates which items contribute to each score, with some items contributing to more than one score.

APOE, Family History and Cognitive Data

APOE genotype was expressed as a binary categorical variable, with participants classified as carriers (one or more ε4 alleles present) or non-carriers (no ε4 allele present). Family history of AD was determined by a multidisciplinary diagnostic consensus panel, as previously described (Sager, Hermann and La Rue 2005). Positive family history of AD was defined as having one or both parents with autopsy-confirmed or probable AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann *et al.* 1984, 2011).

Table 1. Sleep Scales

	ESS	Sleep Disturbance	Snoring	Waking short of breath	Sleep Adequacy	Somnolence	Sleep Problems Index I	Sleep Problems Index II	Sleep Quantity
EPWORTH SLEEPINESS SCALE (ESS)									
How likely are you to doze off or fall asleep in the following 8 situations? [a]	O								
MOS SLEEP SCALE									
During the past 4 weeks…									
1. How long did it usually take for you to fall asleep? [b]		O						O	
2. On the average, how many hours did you sleep each night? [c]									O
How often did you… [d]									
3. feel that your sleep was not quiet (moving restlessly, feeling tense, speaking, etc., while sleeping)?		⊙						⊙	
4. get enough sleep to feel rested upon waking in the morning?					⊙		O	O	
5. awaken short of breath or with a headache?				⊙			⊙	⊙	
6. feel drowsy or sleepy during the day?						⊙		⊙	
7. have trouble falling asleep?		⊙					⊙	⊙	
8. awaken during your sleep time and have trouble falling asleep again?		⊙					⊙	⊙	
9. have trouble staying awake during the day?						⊙	⊙	⊙	
10. snore during your sleep?			⊙						
11. take naps (5 minutes or longer) during the day?						⊙			
12. get the amount of sleep you needed?					⊙		O	⊙	

[a] Responses were on a 4-point scale ranging from 0 = "would never doze" to 3 = "high chance of dozing". Responses were summed to produce a total score ranging from 0 to 24, with higher scores indicating greater daytime sleepiness. [b] Possible responses were 15 minute increments from 1 = "0-15 minutes' to 5 = "More than 60 minutes". [c] Responses were free-entry. [d] Responses were on a 6-point scale ranging from 1="All of the time" to 6="None of the Time". [b,c,d] Responses were converted to a 0-100 scale, then summed to give scores. O indicates item included in scale, ⊙ indicates that item was reversed before computing scale.

Participants completed a comprehensive battery of standard psychometric tests and health and lifestyle questionnaires (Sager, Hermann and La Rue 2005). Here we report measures known to be associated with AD and/or sleep function. Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff 1977), global cognitive function was assessed with the Mini Mental State Exam (MMSE) (Folstein, Folstein and McHugh 1975), episodic memory was assessed with the Rey Auditory Verbal Learning Task

(AVLT) (Spreen and Strauss 1998) and executive function was assessed with the Trail Making Tests A and B (Reitan and Wolfson 1993) and the Digit Symbol Substitution subtest of the Wechsler Adult Intelligence Scale (WAIS-III) (Wechsler 1997).

Statistical Analysis

We used multiple regression to assess the relationship between self-reported sleep and regional amyloid load, quantified as PiB DVR. Separate models were tested for each possible sleep score and ROI combination, with sleep as the predictor of interest and PiB DVR as the outcome, using SPSS version 22 (IBM Corporation, Armonk, New York). Because sleep and amyloid can be affected by age, sex, APOE4 genotype, family history of AD and body mass index (BMI) these were included as covariates. Age was taken from the PET scan. It has previously been shown that the effect of sleep disordered breathing on cognition depends on APOE genotype (Nikodemova *et al.* 2013), therefore for each possible combination of sleep score and ROI we tested a regression model that included a term for the interaction of sleep score with binary APOE4 status. Case analyses identified no outliers in need of removal. To explore the difference between ESS and MOS somnolence scores we used Pearson's correlation to test the relationship between ESS and the 3 sub-items of the MOS somnolence score. Results were considered statistically significant when $p<.05$.

RESULTS

Participant Characteristics

Participant characteristics are summarized in Table 2. The mean age was 62.4 years (SD = 5.7, range = 50-73) at the time of the PET scan. Mean interval between PET scan and questionnaire completion was 0.69 (SD.98) years, and the results did not change when interval was added as a covariate. The sample was enriched for AD risk factors: 34 (34.7%) were APOE4 carriers, 74 (75.5%) had one or both parents with AD. Four participants had MMSE scores between 23 and 26, within the range of possible mild cognitive impairment. The remaining 94 participants had

MMSE scores ≥27. Six participants had a CES-D score of 15-21, indicating possible mild-moderate depressive symptoms. One participant had a CES-D score of 27, indicating possible major depressive symptoms.

Table 2. Participant characteristics (n=98).

Variable	Data (n=98)
Age at PiB PET scan, y	62.4 (5.7; 50-73)
Age at sleep assessment, y	63.0 (5.6; 51-73)
Interval between PiB PET scan and sleep assessment, y	.69 (.98; 0-3.7)
Female, %	67.3
APOE4 positive, %	34.7
FH positive, %	75.5
Maternal FH positive, %	52
BMI, kg/m^2	28.7 (5.7)
Education, y	16.582 (2.832; 12-25)
CES-D	5.78 (5.48; 0-27)
MMSE	29.31 (1.22; 23-30)
AVLT total	50.21 (8.66; 30-67)
AVLT delayed recall	10.36 (2.96; 0-15)
Trails A Time*	10.11 (2.18; 5-17)
Trails B Time*	10.26 (2.51; 6-17)
Digit Symbol*	13.35 (2.1; 9-19)

All values are mean (SD; range) except where indicated. APOE4, the epsilon 4 allele of the apolipoprotein E gene; FH, family history of Alzheimer's disease; BMI, Body Mass Index, CES-D, Center for Epidemiological Studies Depression Scale; MMSE, Mini-Mental State Exam; AVLT, Auditory Verbal Learning Test; *scaled for age and gender.

Association of Self-Reported Sleep Characteristics with Regional Amyloid Load

After adjusting for covariates, poorer sleep was significantly associated ($p<.05$) with greater amyloid burden in most of the ROIs examined, across multiple sleep measures, with effect sizes ranging from small to medium (partial $\eta^2 = .04$-$.09$). Sleep scores that were significantly associated with PiB DVR are in Table 3, other scores are in Supplemental Table 2. Note that beta coefficients appear small because responses to the MOS questionnaire were converted from a 6-point to 100-point scale for scoring. Data for 3 representative sleep score - ROI combinations are plotted in Figure 2.

Sleep Adequacy

Sleep Adequacy is derived from two questions; one asks whether respondents are getting the amount of sleep they need and the other asks whether respondents are getting enough sleep to feel rested. Less adequate sleep was associated with greater amyloid burden in the angular gyrus, anterior and posterior cingulate, frontal medial orbital gyrus, precuneus, supramarginal gyrus, temporal middle gyrus and temporal superior gyrus, and averaged across all 8 ROIs (Table 3).

Table 3. Association between self-reported sleep and regional amyloid burden

		Sleep Adequacy					Sleep Problems Index I					Somnolence				
ROI		B	SE	t	p	η^2_p	B	SE	t	p	η^2_p	B	SE	t	p	η^2_p
Angular Gyrus	L	-.002	.001	-1.948	.055	.041	.002	.002	1.409	.162	.022	.003	.002	2.282	**.025**	.055
	R	-.003	.001	-2.910	**.005**	.087	.004	.002	2.299	**.024**	.056	.004	.001	2.461	**.016**	.064
Cingulum Anterior	L	-.003	.001	-2.565	**.012**	.069	.004	.002	2.176	**.032**	.051	.004	.002	2.258	**.026**	.054
	R	-.004	.001	-2.819	**.006**	.082	.004	.002	2.213	**.029**	.052	.004	.002	2.146	**.035**	.049
Cingulum Posterior	L	-.003	.001	-2.536	**.013**	.067	.004	.002	2.155	**.034**	.050	.002	.002	1.300	.197	.019
	R	-.002	.001	-2.373	**.020**	.059	.003	.002	2.113	**.037**	.048	.002	.001	1.291	.200	.018
Frontal Med Orb	L	-.003	.001	-2.843	**.006**	.083	.004	.002	2.234	**.028**	.053	.004	.002	2.721	**.008**	.077
	R	-.004	.001	-2.896	**.005**	.086	.004	.002	2.219	**.029**	.052	.004	.002	2.196	**.031**	.051
Precuneus	L	-.003	.001	-2.461	**.016**	.064	.004	.002	2.154	**.034**	.050	.003	.002	2.090	**.040**	.047
	R	-.003	.001	-2.823	**.006**	.082	.004	.002	2.331	**.022**	.058	.003	.002	2.020	**.046**	.044
Supra-marginal	L	-.001	.001	-1.472	.144	.024	.002	.001	1.180	.241	.015	.003	.001	2.246	**.027**	.054
	R	-.002	.001	-2.473	**.015**	.064	.003	.001	1.856	.067	.037	.003	.001	2.080	**.040**	.046
Temporal Mid	L	-.001	.001	-1.542	.127	.026	.001	.001	1.068	.288	.013	.002	.001	2.006	**.048**	.043
	R	-.002	.001	-2.067	**.042**	.046	.002	.002	1.353	.180	.020	.002	.001	1.711	.090	.032
Temporal Sup	L	-.001	.001	-.830	.409	.008	.001	.001	.676	.501	.005	.002	.001	1.864	.066	.038
	R	-.002	.001	-1.998	**.049**	.043	.002	.001	1.277	.205	.018	.002	.001	1.663	.100	.030
Mean		-.002	.001	-2.503	**.014**	.066	.003	.002	1.973	*.052*	.042	.003	.001	2.171	**.033**	.050

ROI, regions of interest; B, unstandardized regression coefficient; SE, standard error of the coefficient; p, p-value; η^2_p, partial eta squared. All models were adjusted for age, sex, APOE4, Family history of Alzheimer's disease and body mass index. Statistically significant associations in bold, trends in italics.

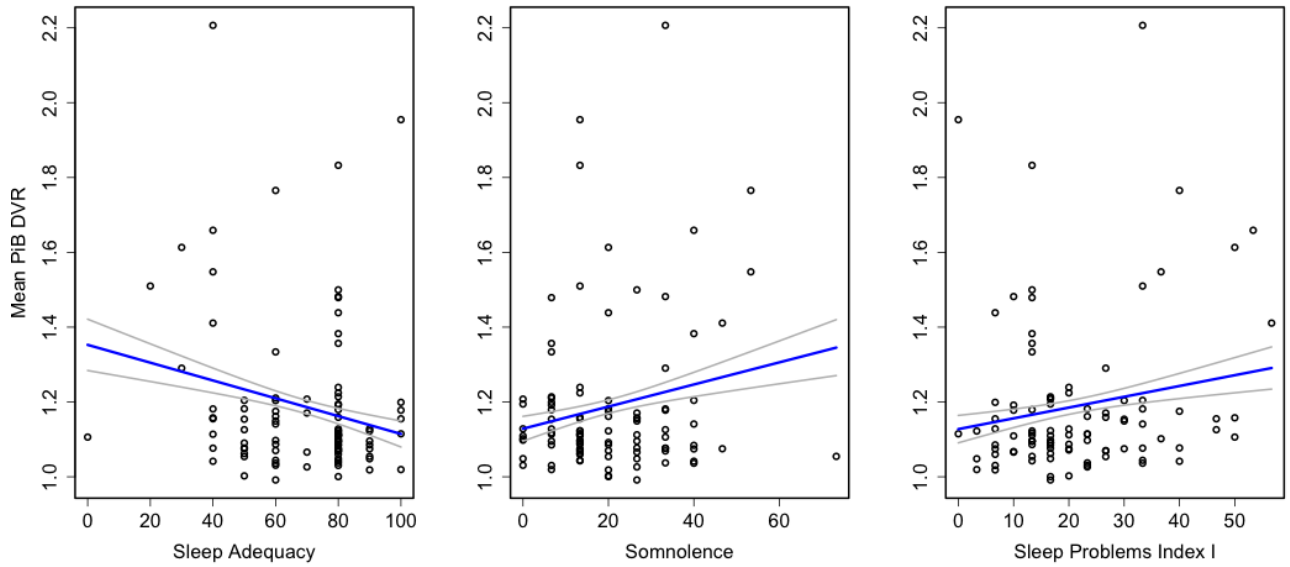

Figure 2. Association between sleep scores and mean PiB DVR. Raw data is plotted, regression line is adjusted for age, sex, APOE4, family history of Alzheimer's disease and body mass index.

Sleep Problems

MOS Sleep Problems Index I is computed from six items probing a range of sleep issues including sleep disordered breathing, insomnia and restfulness. Higher scores were associated with greater amyloid burden in the angular gyrus, anterior and posterior cingulate, frontal medial orbital cortex and precuneus (Table 3). Similar results were observed for Sleep Problems Index II (Supplemental Table 1).

Somnolence and Sleepiness

Participants reporting greater Somnolence on the MOS had greater mean amyloid burden, and regionally in the angular gyrus, anterior cingulum, frontal medial orbital cortex, precuneus, supramarginal gyrus and middle temporal gyrus (Table 3). By contrast, there was no association between PiB DVR and sleepiness assessed by ESS (Supplemental Table 2). We explored the differences between ESS and MOS Somnolence further by testing the three MOS Somnolence sub-items individually against PiB DVR. The sub-item most similar to the ESS, "trouble staying

awake during the day", was not associated with regional amyloid load, whereas feeling drowsy and taking naps were significantly associated with greater amyloid burden in several regions (Table 4). We further examined the relationship between the ESS and MOS Somonolence with Pearson correlation. ESS was significantly correlated with the MOS Somnolence score (r=.27, p=.01) and with 2 of the 3 MOS somnolence sub-items: feeling drowsy (MOS Q6; r=.36, p=.0003) and trouble staying awake during the day (MOS Q9; r=.38, p=.0001) (Table 5). ESS was not correlated with the sub-item taking naps.

Table 4. Association between MOS somnolence sub-items and regional amyloid burden

		Q6: feel drowsy[a]					Q9: trouble staying awake[a]					Q11: take naps[a]				
ROI		B	SE	t	p	η^2_p	B	SE	t	p	η^2_p	B	SE	t	p	η^2_p
Angular Gyrus	L	.002	.001	1.939	*.056*	.041	.001	.002	.906	.367	.009	.002	.001	2.162	**.033**	.050
	R	.002	.001	2.262	**.026**	.054	.002	.001	1.436	.154	.023	.002	.001	1.839	.069	.037
Cingulum Anterior	L	.003	.001	2.026	**.046**	.044	.003	.002	1.638	.105	.029	.002	.001	1.520	.132	.025
	R	.003	.001	2.075	**.041**	.046	.003	.002	1.595	.114	.028	.002	.001	1.287	.202	.018
Cingulum Posterior	L	.002	.001	1.320	.190	.019	.001	.002	.718	.474	.006	.001	.001	.903	.369	.009
	R	.001	.001	1.349	.181	.020	.001	.001	.802	.425	.007	.001	.001	.802	.425	.007
Frontal Med Orb	L	.003	.001	2.472	**.015**	.064	.003	.002	1.629	.107	.029	.002	.001	2.016	**.047**	.044
	R	.003	.001	2.221	**.029**	.053	.003	.002	1.415	.161	.022	.002	.001	1.374	.173	.021
Precuneus	L	.002	.001	1.989	**.050**	.043	.002	.002	1.349	.181	.020	.002	.001	1.422	.158	.022
	R	.002	.001	1.961	*.053*	.041	.002	.002	1.328	.188	.019	.002	.001	1.326	.188	.019
Supra-marginal	L	.001	.001	1.648	.103	.030	.001	.001	1.065	.290	.013	.002	.001	2.256	**.027**	.054
	R	.002	.001	1.944	*.055*	.041	.002	.001	1.336	.185	.020	.001	.001	1.453	.150	.023
Temporal Mid	L	.001	.001	1.685	.095	.031	.001	.001	.782	.436	.007	.002	.001	1.938	*.056*	.040
	R	.002	.001	1.676	.097	.031	.001	.001	.850	.398	.008	.001	.001	1.306	.195	.019
Temporal Sup	L	.001	.001	1.424	.158	.022	.001	.001	.610	.543	.004	.002	.001	2.024	**.046**	.044
	R	.001	.001	1.562	.122	.027	.001	.001	.921	.360	.009	.001	.001	1.265	.209	.018
Mean		.002	.001	1.998	**.049**	.043	.002	.001	1.259	.211	.017	.002	.001	1.635	.106	.029

ROI, regions of interest; B, unstandardized regression coefficient; SE, standard error of the coefficient; p, p-value; η^2_p, partial eta squared. All models were adjusted for age, sex, APOE4, Family history of Alzheimer's disease and body mass index. Statistically significant associations in bold, trends in italics. [a]How often during the past 4 weeks did you… (Q6) feel drowsy or sleepy during the day? (Q9) have trouble staying awake? (Q11) take naps (5 min or longer) during the day?

Table 5. Correlation of MOS and ESS sleepiness reports.

MOS Qs	**Correlation with ESS**	
How often during the past 4 weeks did you…	**r**	**p**
Q6 *…feel drowsy or sleepy during the day?*	.36	**.00032**
Q9 *…have trouble staying awake during the day?*	.38	**.0001**
Q11 *…take naps (5 min or longer) during the day?*	.13	.19
Somnolence *(mean of Qs 6, 9 & 11)*	.27	**.01**

r, Pearson correlation coefficient; p, p-value; statistically significant associations are in **bold**. ESS, Epworth Sleepiness Scale. For 8 situations, asks "How likely are you to doze off or fall asleep in the following situations?" Responses are on a 4 point scale (no chance of dozing, slight chance, moderate chance, definitely would not doze).

Sleep Quantity, Sleep Disturbance and Sleep Disordered Breathing

There were no significant associations between regional amyloid burden and sleep quantity, expressed as a continuous variable (number of hours spent sleeping per night). Amyloid load was not significantly associated with sleep disturbance (defined by the MOS as unrestful sleep, trouble falling and staying asleep) or symptoms of sleep disordered breathing (waking short of breath or with a headache, snoring) (Supplemental Table 2).

Effect of APOE, Depressive Symptoms and Cognitive Status

We found no statistically significant interaction of sleep scales with APOE4 status, age or gender (i.e., the relationship between sleep and PiB DVR did not depend on APOE4 status, age or gender). When CES-D was added as a covariate, the results did not change substantially. Three associations no longer reached significance, but remained as trends ($p<.063$): sleep adequacy with right middle temporal gyrus and left superior temporal gyrus PiB DVR, and sleep problems index I with left and right posterior cingulate PiB DVR.

When the 4 participants with MMSE <27 were excluded, the general pattern of results did not change substantially. Significant associations ($p<.05$) that became trends ($p<.065$) were between somnolence and PiB DVR in the right precuneus, right supramarginal gyrus and left middle temporal gyrus, between sleep adequacy and right temporal middle and superior gyri PiB DVR, and between sleep problems index I and left precuneus PiB DVR.

DISCUSSION

Our objective was to determine whether sleep problems and/or specific aspects of self-reported sleep are associated with amyloid burden during late mid-life, when interventions to prevent AD may be most effective. Compared to the only previous study of sleep and direct measures of amyloid deposits (Spira *et al.* 2013), we studied an age group 15 years younger, and examined more brain regions and more sleep domains. We found that in cognitively healthy late middle-aged adults, greater regional amyloid burden was associated with self-report of less adequate sleep, greater sleepiness, more sleep problems and more napping. There was no apparent association between amyloid burden and sleep duration, sleep disturbance or symptoms of sleep disordered breathing.

Perceived Sleep Quality, Sleepiness and Amyloid

The sleep measure most consistently associated with increased amyloid burden across brain regions was a perception of less adequate sleep, derived from items asking whether participants were getting the amount of sleep they needed and enough sleep to feel rested. Higher amyloid levels were also associated more sleep problems. These findings are consistent with those in elderly adults, in whom worse perceived sleep quality was associated with greater PiB amyloid burden (Spira *et al.* 2013).

Greater amyloid burden was associated with a higher MOS somnolence score but not a higher score on the Epworth Sleepiness Scale, which may appear surprising initially. However, the ESS has been shown to underestimate daytime sleepiness in adults over 65 years (Onen *et al.* 2013). Furthermore, the scales assess distinct constructs: feeling sleepy (MOS) vs. propensity for falling asleep (ESS) (Kim and Young 2005). The ESS asks about the propensity to fall asleep while performing other activities (e.g. talking to someone, reading, driving) whereas the MOS somnolence score assesses feeling sleepy without falling asleep, voluntary napping, and trouble staying awake during the day. Indeed, ESS did not correlate with the MOS sub-item

'taking naps'. The MOS somnolence score may capture a broader definition of perceived sleepiness or sleep inadequacy that does not require falling asleep. Our findings suggest that greater amyloid burden is associated with drowsiness but not involuntary daytime sleep. It also highlights the importance of assessing a range of self-reported sleep characteristics, given that sleep is a complex, multidimensional concept.

Sleep Quantity and Amyloid

There was no association between self-reported sleep duration and amyloid burden. This is consistent with previous studies in older adults using objective measures of sleep duration (polysomnography, actigraphy) and amyloid (Aβ42) in cerebrospinal fluid (Ju *et al.* 2013) or plaques (Spira *et al.* 2014). In contrast, Spira et al. (2013) found that, in an older age group, self-reported shorter sleep was correlated with greater amyloid burden in the precuneus and averaged across the cortex. Whereas Spira et al measured sleep duration with a 4-category scale, we treated sleep duration as a continuous variable (number of hours slept) because criteria for optimal sleep quantity are widely debated in the literature. Furthermore, categorization of continuous variables in regression analyses reduces power and increases the likelihood of false positives (Royston, Altman and Sauerbrei 2006). We recoded our data according to the categorical scale of Spira et al (≤5; >5 to 6; >6 to 7; >7 hours) and again found no association between sleep duration and amyloid in our sample (data not shown). However, we cannot discount the possibility that sleep duration may impact amyloid deposition differently in older vs. middle aged subjects.

Sleep Disorders and Amyloid

We found no association between reports of symptoms of sleep disordered breathing and amyloid burden, consistent with a prior finding of no relation of amyloid to reports of waking several times during the night in an elderly cohort (Spira *et al.* 2013). This is surprising, given that several lines of evidence link sleep disordered breathing with AD. Sleep disordered

breathing is more prevalent in dementia than the general population (Frohnhofen and Roffe 2012), increases risk of developing AD (Yaffe *et al.* 2011) and is correlated with AD markers in human cerebrospinal fluid (amyloid and tau) (Osorio *et al.* 2013, 2014). In rodents, sleep disruption and intermittent hypoxia (features of sleep disordered breathing) increase amyloid production and deposition (Kang *et al.* 2009; Shiota *et al.* 2013b). We chose not to consider diagnosis of sleep disorders in this analysis because obstructive sleep apnea is underdiagnosed (Kapur *et al.* 2002). We measured self-reported symptoms, however sleep disordered breathing can be present without awareness or endorsement of symptoms (Halász *et al.* 2004; Gooneratne and Vitiello 2014). Therefore it is likely that some participants who did not report symptoms of sleep-disordered breathing actually did suffer from the disorder. Similarly, we found no association between brain amyloid and reports of insomnia-type symptoms (trouble falling and staying asleep). This is consistent with a study using a similar measure (self report of trouble falling asleep, trouble staying asleep and use of sleep medications), which found no association between disturbed sleep and Aβ42 in plasma, even though those reporting troubled sleep had a ~1.5 higher risk of developing AD (Benedict *et al.* 2014). In contrast, behavioral measurement of sleep continuity (actigraphy) found that those with pathological CSF Aβ42 levels had significantly lower sleep efficiency. Although sleep disordered breathing and insomnia were not individually associated with amyloid, both contributed to the sleep problems index, which was positively associated with greater amyloid burden. These findings highlight the need for further studies using physiological measures of sleep, breathing and hypoxia to clarify their relationship with amyloid deposition. Self-reported sleep measures remain important, given that perceptions of disturbed sleep are what drive patients to seek clinical evaluation for sleep disorders.

Possible Mechanisms Linking Sleep and Amyloid

Because this study was cross-sectional, we cannot determine whether poor sleep drives amyloid deposition or vice versa. Studies in mice suggest the relationship may be bidirectional. Sleep restriction increased amyloid plaque burden (Kang *et al.* 2009), and chronic intermittent hypoxia during sleep (a feature of obstructive sleep apnea) increased CSF Aβ42 (Shiota *et al.* 2013b). Conversely, rising plaque burden was accompanied by disrupted sleep, and immunization against plaque formation preserved sleep (Roh *et al.* 2012).

Aβ accumulation arises from an imbalance between Aβ production and clearance, and plaque formation is dependent on regional Aβ concentrations (Thal *et al.* 2006). Sleep disruption may affect several steps in the process of amyloid plaque formation. Amyloid is released during synaptic activity (Cirrito *et al.* 2005) and brain regions with greater neuronal activity show greater Aβ concentrations in interstitial fluid, and subsequently more plaque formation (Bero *et al.* 2011). Synaptic activity and synaptic strength are progressively decreased during sleep (Vyazovskiy *et al.* 2009; Tononi and Cirelli 2012). Therefore sleep disruption could chronically elevate neuronal activity, thereby increasing amyloid release. The resulting accumulation of extracellular amyloid would result in greater aggregation and plaque formation.

Aβ levels in CSF follow a diurnal pattern (Kang *et al.* 2009), with clearance greatest during sleep (Xie *et al.* 2013). In healthy men (40-60 years), one night of sleep deprivation abolished the overnight decline in CSF Aβ42 (Ooms *et al.* 2014). More wakefulness during the sleep period (i.e. lower sleep efficiency) may reduce Aβ clearance, leading to its accumulation and then aggregation into plaques. Consistent with this hypothesis, a study of older adults (mean 65.6 years) found lower sleep efficiency (measured objectively with actigraphy) and more napping (measured by sleep diary) in the group with lower CSF Aβ42, indicative of Aβ42 sequestration into plaques (Ju *et al.* 2013).

In addition to sleep disruption promoting amyloid deposition, amyloid may affect sleep by impairing the function of sleep-active brain regions and networks. Sleep is characterized by electrical oscillations in cortico-cortical and thalamo-cortical neural assemblies that directly contribute to neural plasticity and daytime cognitive function (Poe, Walsh and Bjorness 2010). Aβ disrupts synaptic transmission (Wang *et al.* 2009), network oscillations (Palop and Mucke 2010) and functional connectivity (Sheline *et al.* 2010; Bero *et al.* 2012). Amyloid may impair the restorative functions of sleep oscillations, leading to perceptions of inadequate sleep and impaired daytime cognitive function.

Limitations

Strengths of this study include the assessment of multiple aspects of sleep, regional amyloid binding and a well-characterized cohort in midlife, an age range that may be optimal for interventions. Limitations discussed above include the use of self-report, which decreased our ability to detect sleep disorders such as sleep disordered breathing. There were multiple comparisons, however the likelihood of Type I error was minimized by restricting analyses to regions of interest chosen a priori based on published literature. The consistent relationship of mean PiB DVR (averaged across all 8 ROIs) to sleep factors adds confidence to the findings. Although the PET scan and questionnaire were completed on separate days, sleep disorders are typically chronic, and the sleep questionnaire asked for symptoms over the previous 4 weeks. Indeed, results did not change when interval was included as a covariate (data not shown). The study was cross-sectional and observational, therefore we cannot determine causality. However, this study establishes a link between sleep and amyloid in mid-life, prior to the onset of AD symptoms.

Implications

No adequate treatments exist to reverse or prevent AD (Schneider *et al.* 2014b). Effective treatments already exist for optimizing sleep, and treating sleep disorders in AD patients

improves cognition, although dementia is not resolved (Ancoli-Israel *et al.* 2008). Our findings suggest that earlier interventions to improve sleep quality and to treat sleep disorders could potentially impact AD progression by reducing amyloid pathology. Additionally, sleep characteristics that are modified by amyloid may provide early biomarkers of preclinical AD and may be useful for diagnosis, prognosis and for monitoring effectiveness of treatments. Prospective longitudinal studies and randomized control trials in cohorts at risk for AD are needed to determine which aspects of sleep are the most useful as treatment targets or disease markers.

Greater amyloid burden was associated with perceptions of inadequate sleep, daytime sleepiness and napping, but not with reported sleep amount or symptoms of sleep disordered breathing or insomnia. It will be important for future studies to include physiological measures of the signs and symptoms of sleep disorders. However, healthy sleep is more than the absence of sleep disorders; it can be broadly defined as patterns of sleep-wakefulness that produce physical, mental and social well-being (Buysse 2014). Healthy sleep may take different forms across individuals and cultures. For example, sleep duration and cognitive vulnerability to sleep restriction vary between individuals in a trait-like manner (Van Dongen *et al.* 2004; Rupp, Wesensten and Balkin 2012), and across cultures sleep patterns vary from highly consolidated western schedules to siestas or biphasic schedules (Ekirch, 2005; Worthman and Melby, 2002). While it is essential to identify mechanisms in order to effectively target interventions, it is also important to recognize that our understanding of sleep function and regulation continues to evolve. For example it has become increasingly clear in recent years that, rather than being a global state, sleep can be considered a localized phenomenon, taking place independently in discrete neural circuits and even individual cells (Nir *et al.* 2011; Fisher and Vyazovskiy 2014). Therefore perceptions of inadequate sleep may capture defective sleep processes that we have yet to identify on a physiological level. Our finding that neuropathology is associated with

perceptions of inadequate sleep, but not with reported sleep duration or symptoms of sleep disorders highlight the importance of maintaining a broader view of sleep health at this time, when sleep's role in AD pathology is only beginning to be uncovered. For example, rather than considering absolute amounts of sleep, it may be more informative to assess whether individuals are getting less sleep than they need, which is captured by questions addressing sleep adequacy.

Conclusion

We found that self-reported sleep quality, but not quantity, was associated with amyloid plaques in brain regions typically affected in AD. These relationships were present in middle-aged adults who are currently cognitively healthy, therefore sleep may be useful during the preclinical phase of AD as a biomarker or modifiable risk factor to prevent or delay AD. Future work will need to clarify which aspects of sleep are most strongly related to amyloid and other markers of AD pathology, and the mechanisms linking sleep and AD progression.

SUPPLEMENTAL MATERIAL

Supplemental Table 1 Associations between Sleep Problems Index II and regional amyloid burden

ROI		B	SE	t	p	η^2_p
Angular Gyrus	L	.002	.002	1.258	.212	.017
	R	.004	.002	2.217	**.029**	.052
Cingulum Anterior	L	.004	.002	2.091	**.039**	.047
	R	.005	.002	2.143	**.035**	.049
Cingulum Posterior	L	.004	.002	1.978	*.051*	.042
	R	.003	.002	1.906	*.060*	.039
Frontal Med Orb	L	.004	.002	2.292	**.024**	.056
	R	.005	.002	2.199	**.030**	.052
Precuneus	L	.004	.002	2.005	**.048**	.043
	R	.004	.002	2.221	**.029**	.053
Supra-marginal	L	.002	.001	1.144	.256	.014
	R	.003	.002	1.695	.093	.031
Temporal Mid	L	.002	.001	1.095	.277	.013
	R	.002	.002	1.347	.182	.020
Temporal Sup	L	.001	.001	.785	.435	.007
	R	.002	.002	1.198	.234	.016
Mean		.003	.002	1.894	.061	.039

ROI, regions of interest; B, unstandardized regression coefficient; SE, standard error of the coefficient; p, p-value; η^2_p, partial eta squared. All models were adjusted for age, sex, APOE4, Family history of Alzheimer's disease and body mass index.

Supplemental Table 2 Non-significant associations between sleep and regional amyloid burden

		ESS					Sleep Disturbance					Snoring					Waking Short of Breath					Sleep Quantity				
ROI		B	SE	t	p	η^2_p	B	SE	t	p	η^2_p	B	SE	t	p	η^2_p	B	SE	t	p	η^2_p	B	SE	t	p	η^2_p
Angular Gyrus	L	.000	.007	-.024	.981	.000	.000	.001	.136	.892	.000	.000	.001	-.256	.799	.001	.000	.002	.023	.981	.000	-.016	.020	-.772	.442	.007
	R	.001	.006	.091	.928	.000	.001	.001	.905	.368	.009	.000	.001	.035	.972	.000	.000	.001	-.027	.979	.000	-.025	.019	-1.290	.201	.019
Cingulum Anterior	L	.002	.008	.233	.817	.001	.001	.002	.787	.434	.007	.000	.001	.342	.733	.001	.001	.002	.612	.542	.004	-.033	.024	-1.393	.167	.022
	R	.006	.008	.698	.487	.005	.001	.002	.696	.489	.005	.001	.001	.572	.569	.004	.001	.002	.756	.452	.006	-.034	.024	-1.409	.162	.023
Cingulum Posterior	L	-.006	.007	-.836	.405	.008	.001	.001	1.127	.263	.014	.000	.001	.310	.757	.001	.000	.002	.094	.925	.000	-.023	.021	-1.092	.278	.014
	R	-.003	.006	-.506	.614	.003	.001	.001	1.147	.254	.015	.001	.001	1.386	.169	.022	.000	.001	-.226	.822	.001	-.021	.019	-1.119	.266	.014
Frontal Med Orb	L	.003	.007	.368	.713	.002	.001	.001	.882	.380	.009	.000	.001	.130	.897	.000	.001	.002	.323	.748	.001	-.030	.021	-1.464	.147	.024
	R	.003	.008	.413	.681	.002	.001	.002	.739	.462	.006	.000	.001	.273	.786	.001	.001	.002	.740	.461	.006	-.040	.023	-1.687	.095	.032
Precuneus	L	-.002	.007	-.338	.736	.001	.001	.001	.858	.393	.008	.000	.001	.397	.693	.002	.000	.002	.243	.808	.001	-.028	.021	-1.356	.179	.021
	R	-.002	.007	-.331	.742	.001	.001	.001	1.011	.315	.011	.000	.001	.365	.716	.002	.000	.002	.207	.837	.000	-.034	.022	-1.534	.129	.027
Supra-marginal	L	-.002	.005	-.295	.768	.001	.000	.001	.291	.772	.001	.000	.001	.160	.873	.000	.000	.001	-.076	.940	.000	-.011	.016	-.654	.515	.005
	R	.002	.006	.320	.750	.001	.000	.001	.412	.681	.002	.000	.001	-.243	.809	.001	.000	.001	.154	.878	.000	-.019	.017	-1.117	.267	.014
Temporal Mid	L	-.003	.005	-.603	.548	.004	.000	.001	.205	.838	.000	.000	.001	.033	.974	.000	.000	.001	.008	.994	.000	-.003	.016	-.210	.834	.001
	R	-.001	.006	-.186	.853	.000	.000	.001	.369	.713	.002	.000	.001	.220	.826	.001	.000	.001	-.346	.730	.001	-.013	.019	-.714	.477	.006
Temporal Sup	L	-.004	.005	-.844	.401	.008	.000	.001	.201	.841	.000	.000	.001	-.068	.946	.000	.000	.001	.156	.876	.000	-.006	.016	-.377	.707	.002
	R	-.002	.006	-.292	.771	.001	.000	.001	.178	.859	.000	.000	.001	.038	.970	.000	.000	.001	-.283	.778	.001	-.012	.017	-.697	.488	.006
Mean		-.001	.006	-.103	.918	.000	.001	.001	.692	.491	.005	.000	.001	.261	.795	.001	.000	.001	.194	.847	.000	-.022	.019	-1.175	.243	.016

ROI, regions of interest; B, unstandardized regression coefficient; SE, standard error of the coefficient; p, p-value; η^2_p, partial eta squared. All models were adjusted for age, sex, APOE4, Family history of Alzheimer's disease and body mass index.

CHAPTER 3

Poor Sleep Is Associated With CSF Biomarkers Of Amyloid Pathology In Cognitively Normal Adults

Poor sleep is associated with CSF biomarkers of amyloid pathology in cognitively normal adults

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ABSTRACT

Objective: To determine the relationship between sleep quality and cerebrospinal fluid (CSF) markers of Alzheimer's disease (AD) pathology in late mid-life.

Methods: We investigated the relationship between sleep quality and CSF AD biomarkers in a cohort enriched for parental history of sporadic AD, the Wisconsin Registry for Alzheimer's Prevention (WRAP). 101 participants (mean age 62.9 +/-6.2 years, 65.3% female) completed sleep assessments, CSF collection and were cognitively normal. Sleep quality was measured with the Medical Outcomes Study Sleep Scale. CSF was assayed for biomarkers of amyloid metabolism and plaques (Aβ42), tau pathology (P-tau181), neuronal/axonal degeneration (T-tau, NFL), neuroinflammation/astroglial activation (MCP-1, YKL-40) and synaptic dysfunction/degeneration (neurogranin). To adjust for individual differences in total amyloid production, Aβ42 was expressed relative to Aβ40. To assess cumulative pathology, CSF biomarkers were expressed in ratio to Aβ42. Relationships among sleep scores and CSF biomarkers were assessed with multiple regression, controlling for age, sex, time between sleep and CSF measurements, and CSF assay batch.

Results: Worse subjective sleep quality, more sleep problems and daytime somnolence were associated with greater AD pathology, indicated by lower CSF Aβ42/Aβ40 and higher t-tau/Aβ42, p-tau/Aβ42, MCP-1/Aβ42 and YKL-40/Aβ42. There were no significant associations between sleep and NFL or neurogranin.

Conclusions: Self-report of poor sleep was associated with greater AD-related pathology in cognitively healthy adults at risk for AD. Effective strategies exist for improving sleep, therefore sleep health may be a tractable target for early intervention to attenuate AD pathogenesis.

INTRODUCTION

To delay or prevent dementia due to Alzheimer's disease (AD) it is critical to identify modifiable risk factors. Sleep quality is a promising target for intervention during the preclinical phase of AD, when pathogenesis has begun but cognition is still intact (Jack *et al.* 2013). Sleep is associated with AD brain pathophysiology, including amyloid mis-metabolism and deposition, tau hyperphosphorylation and aggregation, and neuronal and synaptic dysfunction and degeneration. We previously found that self-report of poor sleep was associated with greater brain amyloid burden, as measured by PET with Pittsburgh Compound B (PiB) (Sprecher *et al.* 2015). In young men sleep deprivation diminished the diurnal fluctuation of CSF amyloid levels (Ooms *et al.* 2014). In rodent models of AD sleep restriction alters amyloid metabolism and tau phosphorylation, (Kang *et al.* 2009; Xie *et al.* 2013; Di Meco, Joshi and Praticò 2014) and tau-deficient mice show disturbed sleep architecture (Cantero *et al.* 2010). Maintenance of axons (Cirelli 2009) and synapses occurs during sleep (Maret *et al.* 2011), and sleep loss elevates inflammation and microglial activation (Faraut *et al.* 2012).

Preclinical AD brain pathology can be detected via CSF biomarkers, however little is known of their relationship with sleep. CSF amyloid-β42 (Aβ42, a marker of amyloid deposition) may be associated with sleep disordered breathing (Ju *et al.* 2016) and sleep fragmentation (Ju *et al.* 2013). However, less is known about other markers of AD pathophysiology. Furthermore, CSF biomarkers combined in ratios have superior diagnostic and prognostic power compared to single biomarkers (Janelidze *et al.* 2016; Racine *et al.* 2016). Therefore we examined the relationship between self-reported sleep and CSF biomarkers of amyloid deposition and plaque formation (Aβ42), tau phosphorylation state and tau pathology (p-tau), axonal degeneration (t-tau, NFL), neuroinflammation (MCP-1, YKL-40) and synaptic dysfunction/degeneration (neurogranin) in cognitively healthy adults in late middle-age.

METHODS

Participants and Study Design

Participants were drawn from a longitudinal cohort enriched with parental history of AD, the Wisconsin Registry for Alzheimer's Prevention (WRAP) (Sager, Hermann and La Rue 2005). Beginning in 2001, 1500+ WRAP participants were recruited from the community via advertisements and word of mouth and were aged 40-65 years at study entry. Participants underwent extensive cognitive testing and medical history assessment at baseline, 4 years later and every 2 years subsequently at the University of Wisconsin-Madison. Beginning in 2010, participants were sequentially recruited by letter and phone into sub-studies that included CSF collection. Recruitment into CSF sub-studies was based on temporal proximity to recent or upcoming evaluations for the parent study (average interval was 0.84 years), as well as self-reported interest in sub-studies that were advertised at WRAP participant events. All WRAP participants were included in the present analysis who had assayed CSF samples, completed sleep questionnaires, and were cognitively normal. When CSF was available from multiple time-points, the data from the CSF sample collected closest to the sleep questionnaire was used.

Standard Protocol Approvals, Registrations and Patient Consents

The study was approved by the University of Wisconsin - Madison Health Sciences Institutional Review Board and participants provided informed written consent.

CSF Collection and Quantification

CSF was collected in the morning (mean 10:22 am ± 1 hour 12 min SD) following a 12-hour fast. Lumbar puncture was performed with a Sprotte 25- or 24- gauge spinal needle at L3/4 or L4/5, using gentle extraction into polypropylene syringes. Approximately 22mL of CSF was gently mixed to remove gradient effects and centrifuged at 2000g for 10 minutes. 0.5 mL aliquots of supernatants were frozen in polypropylene tubes and stored at -80°C. The samples were sent in 2 batches to the University of Gothenburg in Sweden to be assayed. For the

Aβ42/Aβ40 ratio, CSF Aβ42 and Aβ40 were measured by electrochemiluminescence using an Aβ triplex assay (MSD Human Aβ peptide Ultra-Sensitive Kit, Meso Scale Discovery, Gaithersburg, MD). For all other ratios, CSF Aβ42 was quantified with a sandwich enzyme-linked immunosorbent assay (ELISA), as were t-tau and p-tau181 (INNOTEST β-amyloid1-42, hTAU-Ag and Phospho-Tau[181P], respectively; Fujirebio Europe, Ghent, Belgium). Sandwich ELISAs were also used to assay CSF YKL-40 (R&D Systems, Minneapolis, Minn., USA) and NFL (NF-light ELISA kit, UmanDiagnostics AB, Umeå, Sweden). MCP-1 was measured using the Meso Scale Discovery technique (MSD Human MCP-1; Meso Scale Discovery, Gaithersburg, MD, USA). All assays were conducted by board-certified laboratory technicians blinded to participant clinical characteristics. Assays were completed in a single round of analyses using one batch of reagents, yielding intra-assay coefficients of variation below 10%.

CSF measures were selected for this analysis based on their ability to predict subsequent amyloid accumulation, as we have reported previously in an overlapping sample.[13] For statistical analyses, Aβ42 was expressed in ratio to Aβ40 (the more abundant, non-toxic fragment), to assess the pathologic species (Aβ42) while accounting for individual differences in amyloid production. T-tau, p-tau181, NFL, MCP-1, YKL-40 and neurogranin were expressed in ratios to Aβ42 to reflect coincident pathologies and because these ratios have better diagnostic and prognostic power than each species expressed alone.[13,14] CSF Aβ42 decreases as plaque burden increases, whereas the other CSF markers analyzed are elevated when pathology is greater. Therefore lower Aβ42 and Aβ42/Aβ40 indicate greater pathology, while ratios of other CSF markers to Aβ42 indicate greater pathology when they are elevated.[13]

Sleep Assessment

Sleep was assessed with the Medical Outcomes Study (MOS) Sleep Scale and the Epworth Sleepiness Scale (ESS). The MOS Sleep Scale was selected because it assesses multiple sleep domains with low participant burden and good internal consistency (Viala-Danten *et al.*

2008). It gives scores in 6 sleep domains, derived from 12 questions (summarized in Table 1). Responses were elicited for the past 4 weeks using a 6-point scale, then converted to a 0-100 scale before being summed to give sleep scores. Primary analyses focused on scores we previously found to be associated with brain amyloid burden measured by PiB-PET: Sleep Adequacy, Somnolence and Sleep Problems Index (Sprecher *et al.* 2015). Secondary analyses of symptoms of sleep disordered breathing (snoring and waking short of breath) were conducted to better understand the relationship between sleep and CSF biomarkers. Higher scores indicate more of the construct being measured, such that worse sleep is reflected by lower Sleep Adequacy and higher Somnolence and Sleep Problems Index. The ESS was selected because it is a widely used tool in clinical sleep research for assessing daytime sleepiness and sleep propensity, with good internal validity and test-retest reliability (Johns 1992). Participants use a 4-point scale to rate their likelihood of dozing off in 8 common situations, with higher scores indicating greater sleepiness (range 0-24).

***APOE*, Family History and Cognitive Assessment**

Participants were classified as carriers or non-carriers of one or more Apolipoprotein E (*APOE*) ε4 alleles, determined by standard PCR techniques. Positive parental history of AD was defined as having one or both parents with AD as determined by a validated interview (Kawas *et al.* 1994) or autopsy-confirmed or probable AD as outlined by research criteria (McKhann *et al.* 2011). Detailed medical history and phone interviews were conducted to confirm family history negative participants. Global cognitive function was assessed with the Mini Mental State Exam (MMSE) (Folstein, Folstein and McHugh 1975), and learning and memory with the Rey Auditory Verbal Learning Test (Schmidt, M 1996). Clinical diagnosis of cognitively normal was determined by consensus of a multi-disciplinary panel, based on physical exam, medical and social history neuropsychological testing and self- and informant- reports of cognitive and functional status.

Table 1. Medical Outcomes Study Sleep Scale.

	Sleep Adequacy	Somnolence	Sleep Problems Index	Snoring
During the past 4 weeks…				
1. How long did it usually take for you to fall asleep?[a]			○	
2. On the average, how many hours did you sleep each night?[b]				
How often did you…[c]				
3. feel that your sleep was not quiet (moving restlessly, feeling tense, speaking, etc., while sleeping)?			●	
4. get enough sleep to feel rested upon waking in the morning?	●		○	
5. awaken short of breath or with a headache?			●	
6. feel drowsy or sleepy during the day?		●	●	
7. have trouble falling asleep?			●	
8. awaken during your sleep time and have trouble falling asleep again?			●	
9. have trouble staying awake during the day?		●	●	
10. snore during your sleep?				●
11. take naps (5 minutes or longer) during the day?		●		
12. get the amount of sleep you needed?	●		○	

Circles indicate items included in each score. Filled circles indicate item was reversed before computing score. [a]Possible responses to Question 1 were 15-minute increments from 1 = '0-15 min' to 5 = 'more than 60 min'. [b]Responses to Question 2 were free entry. [c]Responses to Questions 3-12 were on a 6-point scale ranging from 1 = 'all of the time' to 6 = 'none of the time'.

Statistical Analysis

Separate multiple regression models were run for each combination of CSF and sleep measures, with CSF as the dependent variable and sleep as the independent variable, with covariates sex, age at CSF sample, CSF assay batch and time between CSF sample and sleep assessment (all mean centered). Due to missing data, the number of participants included in each analysis ranged from 94 to 101 (Table 3). Ratios of CSF values to Aβ42 were log

transformed to achieve normal distributions. Casewise diagnostics did not reveal any outliers (+/- 3 standard deviations from the mean) that warranted removal, based on influence (Cook's distance < .039) or leverage (dfbeta<0.197). To account for multiple comparisons a false discovery rate (FDR) approach was used, which controls error while preserving power by adjusting the p-value criterion for significance based on the number of tests performed (Curran-Everett 2000). All analyses were conducted with IBM SPSS 23.

RESULTS

101 members of the WRAP cohort met criteria for inclusion (completed CSF assays, MOS Sleep Scale and ESS). Participant characteristics are summarized in Table 2. The sample was in late midlife (age 63.76 ± 6.18 years at time of sleep questionnaires), cognitively normal, and enriched with AD risk (73.3% with family history of AD, 29.7% *APOE* ε4+).

Regression results are summarized in Table 3 and significant relationships between CSF biomarkers and sleep scores are plotted in Figure 1. Lower Aβ42/Aβ40 was associated with lower Sleep Adequacy (Figure 1A), indicating that less adequate sleep was associated with greater amyloid pathology. Aβ42/Aβ40 was not significantly associated with Somnolence, Sleep Problems Index or ESS. Elevated NFL/Aβ42 was associated with greater Somnolence (Figure 1B). There was no significant relationship between NFL/Aβ42 and the other sleep measures examined here. T-tau/Aβ42 and p-tau/Aβ42 were significantly higher with lower sleep adequacy and greater Somnolence and Sleep Problems Index (Figures 1C to 1H). Neither T-tau/Aβ42 nor p-tau/Aβ42 was related to ESS. Higher YKL-40/Aβ42 was associated with lower Sleep Adequacy and greater Sleep Problems Index (Figures 1I, 1J). There was no significant relationship between YKL-40/Aβ42 and daytime sleepiness (Somnolence or ESS). Higher MCP-1/Aβ42 was associated with greater Somnolence (Figure 1K) but no other sleep measures. Neurogranin/Aβ42 was not associated with any of the sleep measures. Greater Tau was associated with lower Sleep Adequacy. There were no other significant relationships between

Table 2. Participant Characteristics.

	n	Mean (±SD)
Demographics		
Age at Sleep Qs (y)	101	63.76 (±6.18)
Age at CSF Sample (y)	101	62.95 (±6.21)
Interval from CSF sample to Sleep Qs (y)	101	0.82 (±1.23)
Female (%)	101	65.30
APOE ε4 Positive (%)	99	29.70
Family History Positive (%)	101	73.30
CES-D	101	6.45 (±6.61)
BMI (kg/m^2)	101	28.92 (±6.20)
Cognition		
MMSE	101	29.29 (±.97)
Education (y)	100	16.60 (±2.8)
RAVLT Total	101	51.71 (±7.73)
RAVLT Delayed	101	10.64 (±2.66)
Sleep		
Sleep Adequacy	101	67.33 (±20.78)
Somnolence	100	22.40 (±16.51)
Sleep Problems Index	100	22.01 (±12.75)
Snoring	101	31.09 (±27.64)
ESS	99	6.43 (±3.48)
CSF Amyloid		
Aβ42 (TRIPLEX) (ng/L)	101	686.36 (±405.26)
Aβ42 (INNOTEST) (ng/L)	101	733.32 (±222.20)
Aβ42/Aβ40	101	0.09 (±0.02)
CSF Neural injury and tangles		
T-tau (ng/L)	101	325.95 (±115.63)
P-Tau (ng/L)	100	48.44 (±15.20)
NFL (ng/L)	96	701.92 (±278.40)
Neurogranin (ng/L)	91	376.17 (±169.91)
T-tau/Aβ42 (ng/L)	101	0.50 (±0.32)
P-Tau/Aβ42 (ng/L)	100	0.07 (±0.05)
NFL/Aβ42 (ng/L)	96	1.05 (±0.58)
Neurogranin/Aβ42 (ng/L)	91	0.58 (±0.36)
CSF Inflammation		
MCP-1 (ng/L)	100	447.50 (±143.68)
YKL-40 (ng/L)	99	159786.56 (±54914.49)
MCP-1/Aβ42 (ng/L)	98	0.67 (±0.36)
YKL-40/Aβ42 (ng/L)	99	239.83 (±126.15)

APOE ε4, ε4 allele of apolipoprotein E; CES-D, Center for Epidemiologic Studies Depression Scale; BMI, Body Mass Index; MMSE, Mini Mental Status Exam; Rey Auditory Verbal Learning Test, RAVLT; ESS, Epworth Sleepiness Scale; Aβ, amyloid beta; T-tau, total tau; P-Tau,

phosphorylated tau; NFL, neurofilament light; MCP-1, monocyte chemoattractant protein-1; YKL-40, chitinase-3-like protein 1.

sleep and CSF biomarkers expressed alone (Supplemental Table e-1). There were no significant interactions with *APOE* ε4 status or age. There was no significant association between CSF biomarkers and self-report of sleep disordered breathing symptoms or sleep duration. Depression, Body Mass Index, education, cardiovascular disease and sleep-affecting medications did not explain any additional variance in CSF biomarkers (Table e-3), except for BMI with log(NFL/Aβ42). The relationship between somnolence and log(NFL/Aβ42) remained statistically significant and effect size (model R^2) did not change substantially, after controlling for BMI (Supplemental Data).

DISCUSSION

Self-report of less adequate sleep, greater daytime sleepiness and more sleep problems were associated with CSF biomarkers of amyloid deposition in combination with tau pathology, axonal degeneration and neuroinflammation.

We have previously shown that self-report of poor sleep was associated with greater brain amyloid burden, measured with PiB PET (Sprecher *et al.* 2015). The CSF results reported here mirror the PiB PET findings, and reveal further relationships between sleep and CSF biomarkers of cumulative AD pathology. CSF biomarkers give complimentary information to amyloid PET scans. First, abnormalities may become apparent in CSF before PET (Palmqvist, Mattsson and Hansson 2016). Second, CSF can be collected and assayed using widely available methods, whereas PET remains more expensive and requires substantial infrastructure. Finally, whereas a PET scan assesses only a single form of pathology, CSF can be assayed for multiple pathologic species simultaneously.

Table 3. Results of multiple regression of CSF biomarkers on sleep

	n	β	R^2	R^2 change	p
Aβ42/Aβ40					
Sleep Adequacy	101	.247	.269	.052	***.010***
ESS	99	.166	.241	.024	.087
Somnolence	100	-.070	.221	.004	.463
Sleep Problems	100	-.148	.238	.021	.114
log(T-tau/Aβ42)					
Sleep Adequacy	101	-.310	.167	.083	***.003***
ESS	99	.016	.085	.000	.880
Somnolence	100	.242	.139	.054	***.017***
Sleep Problems	100	.230	.135	.050	***.021***
log(P-tau181/Aβ42)					
Sleep Adequacy	100	-.281	.112	.068	***.007***
ESS	98	-.037	.090	.001	.726
Somnolence	99	.211	.130	.041	***.038***
Sleep Problems	99	.214	.132	.044	***.033***
log(NFL/Aβ42)					
Sleep Adequacy	96	-.136	.272	.016	.164
ESS	94	-.068	.260	.004	.483
Somnolence	95	.228	.304	.048	***.015***
Sleep Problems	95	.135	.273	.017	.148
log(MCP1/Aβ42)					
Sleep Adequacy	98	-.158	.257	.022	.105
ESS	96	-.104	.245	.010	.287
Somnolence	97	.207	.275	.040	**.028**
Sleep Problems	97	.104	.246	.010	.266
log(YKL-40/Aβ42)					
Sleep Adequacy	99	-.242	.222	.050	***.016***
ESS	97	-.064	.175	.004	.529
Somnolence	98	.184	.203	.031	.060
Sleep Problems	98	.208	.213	.041	**.031**
log(Neurogranin/Aβ42)					
Sleep Adequacy	91	-.174	.070	.026	.126
ESS	89	-.086	.050	.007	.451
Somnolence	90	.166	.069	.026	.133
Sleep Problems	90	.097	.052	.009	.376
log(1/Aβ42)					
Sleep Adequacy	101	-.219	.096	.041	**.040**
ESS	99	-.115	.067	.012	.281
Somnolence	100	.117	.068	.013	.262
Sleep Problems	100	.124	.070	.015	.226
T-tau					
Sleep Adequacy	101	-.215	.089	.040	**.045**
ESS	99	.068	.054	.004	.523
Somnolence	100	.183	.081	.031	.078
Sleep Problems	100	.174	.079	.029	.090

Results of multiple regression of CSF biomarkers on sleep measures, controlling for sex, age at CSF sample, CSF assay batch and time between CSF sample and sleep assessment (all mean

centered). n, sample size, β, standardized regression coefficient; R^2, overall model fit; R^2 change, contribution of sleep term to model fit; p, p-value; bold p<.05, bold italics p(FDR)<.05.

CSF biomarker ratios indexing multiple pathologies capture the temporal relationship between pathologies, which is a better indicator of disease stage than absolute levels of individual pathologies because AD is a progressive and multi-factorial neurodegenerative disease (Jack *et al.* 2013). Multi-pathology ratios have superior predictive power for development of neuropathological features of AD (Racine *et al.* 2016) and appear to provide superior diagnostic markers of AD (Janelidze *et al.* 2016). Our findings confirm their utility in the context of sleep, given that multi-pathology biomarkers were associated with more sleep domains than single-pathology markers. In particular, the only single-pathology markers associated with sleep were amyloid and tau, which were associated with lower sleep adequacy. Yet when expressed in ratio to each other they were also associated with daytime somnolence and sleep problems. 1/Aβ42 was associated with sleep scores in a similar but not identical pattern to the ratios, confirming that the ratios capture information that is different from single markers and that our results were not driven by Aβ42 in the denominator. This is further supported by effect sizes, which were greater for CSF ratios than 1/Aβ42.

There is evidence that sleep impacts AD pathology through multiple pathways. Sleep disturbance may promote amyloid plaque formation through increased amyloid production and/or reduced amyloid clearance. Orexin, an important regulator of the sleep-wake cycle, has been shown to drive production and deposition of Aβ in transgenic mice, possibly by promoting wakefulness (Kang *et al.* 2009). It has also been shown in mice that clearance of exogenous amyloid is greater during sleep, as a result of increased glymphatic flow (Xie *et al.* 2013). Thus sleep disruption could promote a build up of soluble amyloid, leading to aggregation into plaques. These hypotheses remain to be tested in humans.

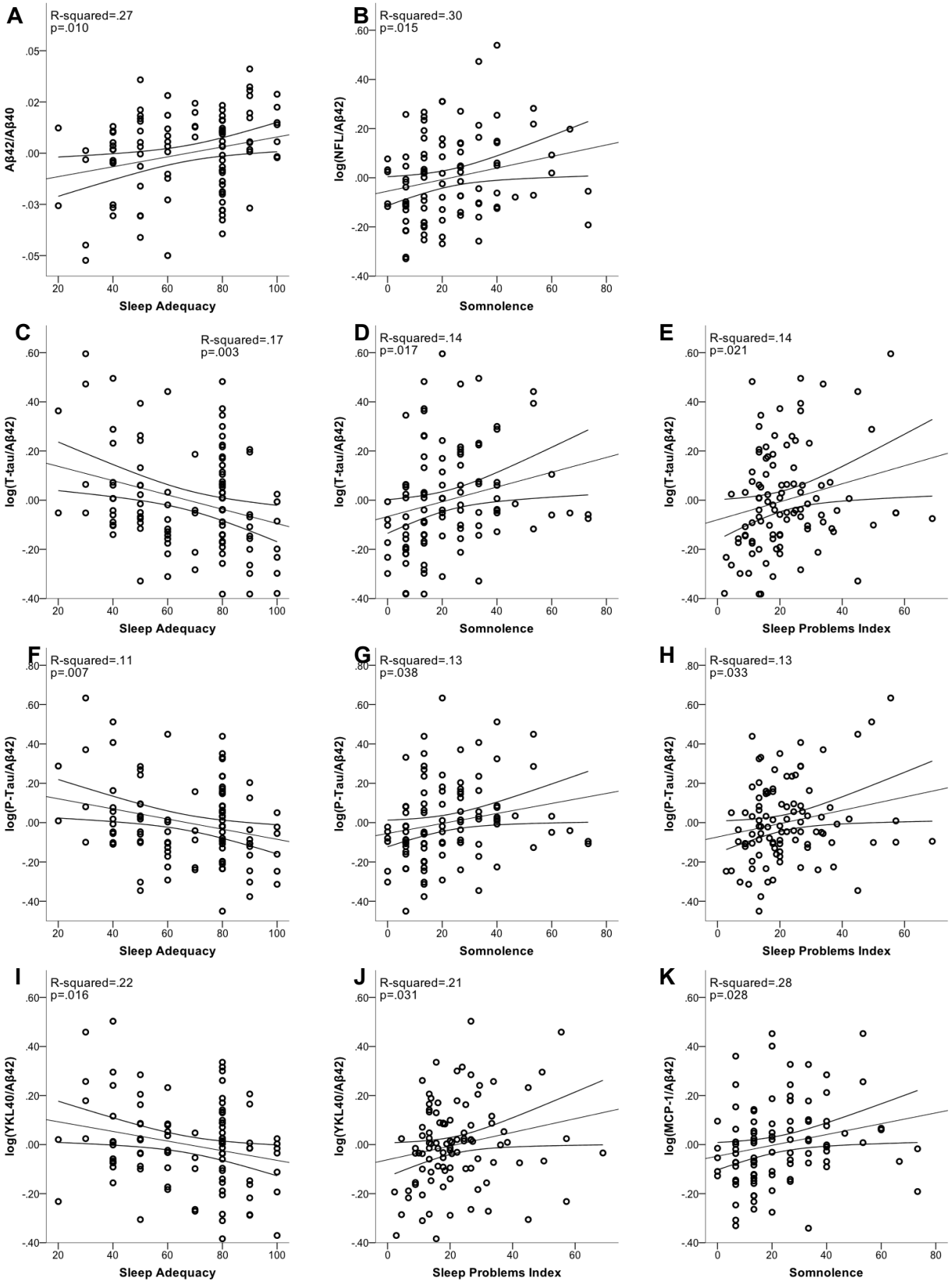
A
R-squared=.27
p=.010
Aβ42/Aβ40
Sleep Adequacy
B
R-squared=.30
p=.015
log(NFL/Aβ42)
Somnolence
C
R-squared=.17
p=.003
log(T-tau/Aβ42)
Sleep Adequacy
D
R-squared=.14
p=.017
log(T-tau/Aβ42)
Somnolence
E
R-squared=.14
p=.021
log(T-tau/Aβ42)
Sleep Problems Index
F
R-squared=.11
p=.007
log(P-Tau/Aβ42)
Sleep Adequacy
G
R-squared=.13
p=.038
log(P-Tau/Aβ42)
Somnolence
H
R-squared=.13
p=.033
log(P-Tau/Aβ42)
Sleep Problems Index
I
R-squared=.22
p=.016
log(YKL40/Aβ42)
Sleep Adequacy
J
R-squared=.21
p=.031
log(YKL40/Aβ42)
Sleep Problems Index
K
R-squared=.28
p=.028
log(MCP-1/Aβ42)
Somnolence

Figure 1. Association of CSF biomarkers with sleep scores.

Regression results and 95% Confidence Intervals are plotted for the significant relationships between CSF biomarkers and sleep scores. CSF measures are adjusted for age, sex, CSF batch and time between CSF sample and sleep questionnaire. Higher sleep scores indicate more of the construct being measured. R^2 is model R^2.

Interestingly, CSF biomarkers of AD pathology were associated with daytime sleepiness indexed by MOS Somnolence but not ESS. We previously observed the same distinction with PET-measured amyloid burden (Sprecher *et al.* 2015). The difference could be due to the nature of the daytime sleepiness: ESS indexes irresistible sleepiness (falling asleep in inappropriate situations such as driving) whereas somnolence may index resistible sleepiness (feeling drowsy and deliberate napping). Orexin, a wake-promoter, is elevated in MCI patients and associated with tau pathology (Liguori *et al.* 2016). It is possible that the sleep disturbance reported here in conjunction with resistible, but not irresistible, sleepiness reflects an abnormal upregulation of the orexin system.

YKL-40 and MCP-1 in CSF are indicative of neuroinflammation and astroglial activation associated with amyloid plaques, with diagnostic and prognostic utility for AD (Craig-Schapiro *et al.* 2010; Fagan and Perrin 2012; Westin *et al.* 2012). Sleep restriction broadly promotes pro-inflammatory cytokines, possibly by disrupting diurnal fluctuations of growth and stress hormone release, and their modulation by slow wave sleep (Faraut *et al.* 2012). Both neuro-toxic and neuro-protective roles have been proposed for YKL-40 and MCP-1 during early stages of amyloid plaque formation (Fagan and Perrin 2012) and further study is required to interpret their relationship with poor sleep.

Degeneration of synapses is an early feature of AD indexed by neurogranin, a post-synaptic protein with diagnostic utility for AD (Tarawneh *et al.* 2016). Maintenance of membranes and synapses is upregulated during sleep (Cirelli 2009; Maret *et al.* 2011), therefore we

hypothesized that sleep disturbance would increase synaptic injury. However we found no relationship between neurogranin and sleep. Synaptic degeneration in AD may be secondary to amyloid and tau pathology (Dorostkar *et al.* 2015), therefore it may be that a relationship between synaptic injury and sleep is not yet detectable in this relatively young healthy cohort.

We did not find a relationship between CSF biomarkers and symptoms of obstructive sleep apnea (OSA). This lack of relationship is surprising, given consistent findings that OSA is a risk factor for dementia, possibly promoting AD pathogenesis via sleep fragmentation (Yaffe *et al.* 2011) and/or hypoxia (Shiota *et al.* 2013b). It is possible however, that OSA impedes the transfer of proteins into the CSF, altering the relationship between CSF concentrations and central nervous system pathology in OSA patients (Ju *et al.* 2016). Alternatively, OSA severity in this sample may have been too low to detect a relationship with CSF, as on average participants rated their snoring frequency as low. Sleep disordered breathing often goes undetected by patients, and subjective reports are not reliable in detecting sleep apnea. Neither snoring nor waking short of breath were significantly correlated with Sleep Adequacy, Somnolence, ESS or each other. This fits with literature showing that snoring and daytime sleepiness add distinct information to predict SDB (Netzer *et al.* 1999). Given that we did not directly test for OSA, further studies measuring sleep and breathing are needed to understand the contribution of sleep disordered breathing to the relationship between poor sleep and CSF biomarkers of AD.

Sleep disturbances may have an impact on AD pathology, but on the other hand, AD pathology may impact sleep quality. Animal models show that increasing amyloid plaque burden is accompanied by increasingly fragmented sleep, which is rescued by elimination of Aβ deposits in the mouse brain by active immunization (Roh *et al.* 2012). In humans there is evidence that amyloid plaques disrupt slow wave sleep, impairing memory consolidation (Mander *et al.* 2013a). Slow wave sleep is important for feeling refreshed (Walsh 2009), therefore our finding

that perceptions of inadequate sleep were related to more AD pathology could reflect an inability to obtain sufficient slow wave sleep as a result of amyloid plaques or possibly elevated presence of oligomeric forms of Aβ.

Limitations of this study include the cross-sectional design and use of subjective sleep measures. There is evidence of bidirectional relationships between sleep and amyloid (Kang *et al.* 2009; Xie *et al.* 2013) that cannot be disentangled by this cross-sectional study design. Longitudinal follow-up within individuals will be important to determine the course of sleep and brain pathology in preclinical AD. Variability in CSF collection time may have contributed some unaccounted for variance in amyloid levels, and mean collection time was 10:22 am, close to a 10 am nadir in CSF amyloid reported by one study (Huang *et al.* 2012). However, the diurnal fluctuation was smaller in older adults, and we found no correlation between CSF collection time and amyloid levels.

This study measured sleep through self-report. Objective sleep measures such as actigraphy and polysomnography would clarify the contribution of sleeping brain activity, breathing and sleep-wake rhythmicity to AD pathogenesis. However, the investigation of self-reported sleep quality is itself an important clinical question. There is substantial inter-individual variability in the impact of sleep disturbance on cognitive and physiologic functioning (Van Dongen *et al.* 2004). Furthermore, sleep health is multidimensional (Buysse 2014), and current objective techniques (e.g. polysomnography and actigraphy) do not fully capture ‘adequate’ sleep (Krystal and Edinger 2008). Thus self-report provides important complementary information on functional outcomes and perceptions.

These results add to a growing body of evidence linking sleep quality with AD neuropathology. Our findings demonstrate that the relationship between sleep and AD pathology is present in late mid-life in the absence of cognitive impairment. Furthermore, we show that the relationship

is detectable using self-report and CSF biomarkers, tools that are relatively inexpensive and accessible, making them appealing for further research in large cohorts and clinical trials. ESS is a simple and widely used measure of sleep dysfunction in clinical sleep research. Given the lack of associations shown here with CSF, and previously with PET biomarkers of AD (Sprecher *et al.* 2015), we recommend that other sleep measures be included in studies of the relationship between sleep and AD. Findings were not altered by race, education, depressive symptoms, BMI or use of sleep medications.

Sleep may be a modifiable risk factor for AD during the earliest stages of the disease, before dementia symptoms appear. Although the effect sizes reported here are modest, it is estimated that delaying AD onset by a mere 5 years would reduce AD cases by 5.7 million and save $367 billion in healthcare spending in the US (Alzheimer's Association 2015). Many effective pharmaceuticals, devices and behavioral interventions are already available in the clinic for improving sleep quality. Follow-up studies are needed to identify the aspects of sleep that are most amenable to modification and most effective in impacting AD pathology, to ultimately delay AD or diminish AD symptoms.

SUPPLEMENTAL DATA

Relationship between sleep and CSF biomarkers

Table e-1. Results of multiple regression of CSF biomarkers on sleep measures, controlling for sex, age at CSF sample, CSF assay batch and time between CSF sample and sleep assessment (all mean centered). n, sample size, β, standardized regression coefficient; R^2, overall model fit; R^2 change, contribution of sleep term to model fit; p, p-value; bold p<.05, bold italics p(FDR)<.05.

	n	β	R^2	R^2 change	p
Aβ42					
Sleep Adequacy	101	.154	.061	.020	.155
ESS	99	.082	.046	.006	.446
Somnolence	100	-.087	.047	.007	.408
Sleep Problems	100	-.047	.042	.002	.654
1/Aβ42					
Sleep Adequacy	101	-.258	.120	.057	**.015**
ESS	99	-.143	.081	.018	.176
Somnolence	100	.137	.080	.017	.186
Sleep Problems	100	.186	.096	.033	.067
P-tau181					
Sleep Adequacy	100	-.177	.068	.027	.102
ESS	98	-.013	.041	.000	.903
Somnolence	99	.157	.064	.023	.135
Sleep Problems	99	.181	.072	.031	.081
NFL					
Sleep Adequacy	96	.045	.238	.002	.649
ESS	94	.019	.237	.000	.849
Somnolence	95	.202	.274	.038	.034
Sleep Problems	95	.028	.237	.001	.769

MCP1					
Sleep Adequacy	100	.000	.478	.000	.998
ESS	98	-.097	.486	.008	.222
Somnolence	99	.094	.486	.008	.229
Sleep Problems	99	-.008	.478	.000	.914
YKL-40					
Sleep Adequacy	99	-.063	.118	.003	.550
ESS	97	.005	.115	.000	.964
Somnolence	98	.127	.130	.015	.213
Sleep Problems	98	.136	.132	.018	.176
Neurogranin					
Sleep Adequacy	91	.004	.021	.000	.972
ESS	89	-.048	.023	.002	.675
Somnolence	90	.110	.032	.011	.326
Sleep Problems	90	-.006	.021	.000	.958

Effect of other potential covariates

Models reported in the body of the manuscript controlled for sex, age at CSF sample, CSF assay batch and time between CSF and sleep assessments. We explored the effect of additional potential covariates on the relationship between sleep and CSF biomarkers.

Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff 1977). The mean score was 6.45 (6.61 SD), range 0-35. 11 participants had CES-D>16, indicating some clinically significant depressive symptoms. CES-D was significantly correlated with Sleep Adequacy, Somnolence, Sleep Problems and ESS, but not with the CSF measures.

14 participants reported use of medications known to affect sleep, summarized in table e-2. For inclusion as a covariate, medication use was coded as present or absent.

Table e-2. Use of medications affecting sleep.

Medications affecting sleep	n Users	Details
Benzodiazepines	*2*	*1 Lorazepam, 1 Diazepam*
Sleep Aids	*4*	*2 melatonin, 2 zolpidem*
Sedating agents, clinical purpose unknown	*9*	*3 diphenhydramine, 1 hydroxyzine, 4 trazodone*

To determine the presence of cardiovascular disease participants were asked “Has a health provider (e.g., doctor, nurse, physician’s assistant or mental health professional) told you that you have any of the following conditions:” Responses were Yes, No, or Don’t know. Listed conditions were heart disease, heart attack, congestive heart failure, recurrent chest pain with exercise, irregular (skipped) heartbeats, coronary bypass surgery, stroke and other heart problem(s). 4 participants reported irregular heartbeats and 1 reported other heart problems (aortic regurgitation, mitral valve prolapse). For inclusion as a covariate, cardiovascular disease was coded as present or absent.

Participant-reported race was 96 White/Caucasian, 1 Asian, 1 Spanish/Hispanic and 3 Black/African American. There was insufficient variability of Race for analysis as a covariate.

To test the effect of additional potential covariates on the relationships between sleep scores and CSF biomarkers, partial correlations were conducted for each CSF biomarker and covariate, controlling for sleep score, sex, age at CSF sample, CSF assay batch and time between CSF and sleep assessments. No additional variance in CSF was explained by depression, years of education, body mass index (BMI), cardiovascular disease, or use of

medications that affect sleep (Table e-3), except for BMI and log(NFL/Aβ42). BMI was significantly correlated with log(NFL/Aβ42), after controlling for somnolence, sex, age at CSF sample, CSF assay batch and time between CSF and sleep assessments (r=-.267, p=.015). The relationship between somnolence and log(NFL/Aβ42) remained statistically significant and effect size (model R^2) did not change substantially, after additionally controlling for BMI (without controlling for BMI: β=.228, R^2=.304, p=.015, controlling for BMI: β=.255, R^2=.363, p=.005).

Table e-3 – Partial Correlation of CSF Biomarkers with potential covariates.

Controlling for sleep score, sex, age at CSF sample, CSF assay batch and time between CSF sample and sleep assessment. Significant correlations (p<.05) are in bold.

		Education (yrs)	BMI (kg/m^2)	Sleep Meds	CVD	CES-D
Sleep Adequacy						
log(1/Aβ42)	r	.060	-.121	.034	-.060	.119
	p	.590	.272	.758	.588	.280
Aβ42/Aβ40	r	-.116	.062	.054	-.091	-.152
	p	.293	.577	.628	.410	.166
log(T-tau/Aβ42)	r	.040	-.128	-.054	.048	.122
	p	.721	.246	.627	.667	.269
log(P-tau/Aβ42)	r	.037	-.136	-.036	.027	.169
	p	.736	.217	.749	.807	.125
log(NFL/Aβ42)	r	.130	**-.281**	-.045	.026	.198
	p	.240	**.010**	.683	.813	.071
log(YKL-40/Aβ42)	r	-.048	-.077	-.095	-.050	.231
	p	.667	.486	.392	.653	.035
log(MCP-1/Aβ42)	r	.134	-.130	.031	-.047	.167
	p	.223	.239	.777	.669	.129
log(Neurogranin/Aβ42)	r	.058	-.086	-.028	-.054	.108
	p	.602	.435	.802	.625	.328
Somnolence						
log(1/Aβ42)	r	.000	-.100	.055	-.080	.137
	p	.999	.369	.622	.470	.218
Aβ42/Aβ40	r	-.066	.034	.029	-.060	-.190
	p	.551	.762	.793	.587	.085
log(T-tau/Aβ42)	r	-.014	-.092	-.019	.015	.155
	p	.903	.408	.865	.893	.163
log(P-tau/Aβ42)	r	-.024	-.106	-.007	.000	.193
	p	.832	.340	.952	.998	.081
log(NFL/Aβ42)	r	.100	**-.267**	-.019	.017	.188

	p	.370	**.015**	.863	.880	.090
log(YKL-40/Aβ42)	r	-.079	-.049	-.065	-.074	.262
	p	.476	.661	.559	.506	.017
log(MCP-1/Aβ42)	r	.048	-.121	.058	-.055	.131
	p	.668	.275	.605	.624	.237
log(Neurogranin/Aβ42)	r	.015	-.072	-.008	-.065	.103
	p	.895	.519	.945	.562	.355
Sleep Problems						
log(1/Aβ42)	r	.021	-.094	.020	-.086	.130
	p	.852	.396	.860	.438	.243
Aβ42/Aβ40	r	-.077	.029	.071	-.058	-.161
	p	.489	.794	.521	.605	.145
log(T-tau/Aβ42)	r	.010	-.085	-.069	.008	.134
	p	.929	.442	.534	.942	.228
log(P-tau/Aβ42)	r	-.003	-.100	-.057	-.006	.169
	p	.982	.368	.609	.954	.127
log(NFL/Aβ42)	r	.130	**-.257**	-.054	.006	.207
	p	.242	**.019**	.631	.955	.060
log(YKL-40/Aβ42)	r	-.068	-.044	-.114	-.078	.227
	p	.541	.692	.304	.486	.039
log(MCP-1/Aβ42)	r	.097	-.108	.016	-.069	.173
	p	.381	.329	.885	.534	.118
log(Neurogranin/Aβ42)	r	.045	-.066	-.031	-.073	.130
	p	.687	.556	.780	.510	.242

CHAPTER 4

High resolution topography of age-related changes in non-rapid eye movement sleep electroencephalography

High resolution topography of age-related changes in non-rapid eye movement sleep electroencephalography

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ABSTRACT

Sleeping brain activity reflects brain anatomy and physiology. The aim of this study was to use high density (256 channel) electroencephalography (EEG) during sleep to characterize topographic changes in sleep EEG power across normal aging, with high spatial resolution. Sleep was evaluated in 92 healthy adults aged 18-65 years old using full polysomnography and high density EEG. After artifact removal, spectral power density was calculated for standard frequency bands for all channels, averaged across the NREM periods of the first 3 sleep cycles. To quantify topographic changes with age, maps were generated of the Pearson's coefficient of the correlation between power and age at each electrode. Significant correlations were determined by statistical non-parametric mapping. Absolute slow wave power declined significantly with increasing age across the entire scalp, whereas declines in theta and sigma power were significant only in frontal regions. Power in fast spindle frequencies declined significantly with increasing age frontally, whereas absolute power of slow spindle frequencies showed no significant change with age. When EEG power was normalized across the scalp, a left centro-parietal region showed significantly less age-related decline in power than the rest of the scalp. This partial preservation was particularly significant in the slow wave and sigma bands.

The effect of age on sleep EEG varies substantially by region and frequency band. This non-uniformity should inform the design of future investigations of aging and sleep. This study provides normative data on the effect of age on sleep EEG topography, and provides a basis from which to explore the mechanisms of normal aging as well as neurodegenerative disorders for which age is a risk factor.

INTRODUCTION

Characterizing brain change across the healthy lifespan is important for understanding the mechanisms of normal aging as well as neurodegenerative disorders for which age is a risk factor. Because changes in sleeping brain activity reflect underlying changes in anatomy and neurophysiology, electroencephalography (EEG) during sleep can be a powerful tool for studying the aging brain. Sleep EEG is impacted by structural characteristics such as the number and health of cells and axons through which signals propagate. For example, power in the delta frequencies (1-4.5 Hz) correlates with the maturation of grey and white matter in adolescents as well as degeneration in elderly adults (Buchmann *et al.* 2011b; Mander *et al.* 2013a; Dubé *et al.* 2015). Sleep EEG also reflects functional properties; for example, power in the slow wave band (1-4.5 Hz) is modulated by synaptic strength (Esser, Hill and Tononi 2007) and synchronous firing (Vyazovskiy *et al.* 2009), key mechanisms of information flow through neural networks (Friston 2011). EEG measures activity arising from neural ensembles on a millisecond timescale, capturing dynamic oscillatory activity that reflects functional connectivity. This fine temporal resolution is a key advantage of EEG over complementary imaging modalities such as magnetic resonance imaging (MRI) and positron emission tomography (PET) that are limited to time units of seconds or minutes. EEG is a particularly useful tool for imaging the brain in older populations, because it can be performed at the bedside and in individuals with contra-indications for MRI, such as metallic implants and claustrophobia. Furthermore, sleep EEG shows high test-retest stability within an individual across short time spans (days-weeks) (Finelli, Achermann and Borbély 2001; De Gennaro *et al.* 2005; Tinguely *et al.* 2006; Israel *et al.* 2012; Lewandowski, Rosipal and Dorffner 2013) and recording during sleep allows the measurement of spontaneous brain activity, free from factors that complicate waking recordings across age ranges, such as movement, variations in attention and the ability to follow instructions. Thus sleep EEG can be used to examine structural and functional changes in dynamic neural networks across the life span.

Age-related alterations in grey and white matter structure, brain metabolism and connectivity show substantial regional variation (Bendlin *et al.* 2010a; Giorgio *et al.* 2010; Raz *et al.* 2010; Lebel *et al.* 2012; Chételat *et al.* 2013). Furthermore, it is becoming increasingly apparent that sleep does not occur uniformly throughout the brain. Rather, it can be a regional phenomenon, such that slow waves and spindles may occur in one brain region independent of activity in other regions (Nir *et al.* 2011), with a topography influenced by prior waking use (Huber *et al.* 2004; Landsness *et al.* 2011). Therefore, we hypothesized that the effect of age on sleep EEG would vary across the scalp. Across childhood, slow wave power shifts from posterior to frontal dominance, and correlates with anatomical cortical maturation (Kurth *et al.* 2010; Buchmann *et al.* 2011b). In adulthood, previous work has suggested that age-related declines in EEG power during sleep are greatest in frontal derivations (Landolt and Borbély 2001; Münch *et al.* 2004; Robillard *et al.* 2010; Carrier *et al.* 2011; Martin *et al.* 2013). However, these studies had low spatial resolution (using only 4-20 electrodes), which could have missed or distorted the pattern of age effects on EEG. Regional variations in activity can be detected with greater accuracy when more electrodes are used; this has been empirically demonstrated using simulated and measured data (Qin, Xu and Yao 2010; Liu *et al.* 2015). Therefore we examined EEG topography with high spatial resolution by recording from 256 electrodes (high density EEG; hdEEG).

Prior studies assessing the effects of aging on sleep EEG topography included age as a binary variable, comparing young adults to older adults, and in the majority of studies the size of each group was small, ranging from 8 to 18. Although some studies have used regression to examine age-related changes in NREM spectral power (Carrier *et al.* 2001), no studies have examined changes in topography throughout the life span. Aging is a progressive, rather than step-wise process, making the treatment of age as a continuous variable a more appropriate method of evaluating the brain changes associated with aging. Furthermore, midlife was largely neglected,

with only one study including adults aged between 40 and 57 years,(Carrier *et al.* 2011) and none including subjects between 30 and 40 years. It is important to investigate midlife as several studies indicate that it is a critical period; brain health at this age may predict resilience or vulnerability to disease in later life. For example, middle aged adults with obstructive sleep apnea (OSA) show greater cognitive deficits than younger adults with equally severe OSA (Alchanatis *et al.* 2008; Ayalon, Ancoli-Israel and Drummond 2010) and mid-life vascular health predicts later development of dementia (Kivipelto *et al.* 2006; Fitzpatrick AL *et al.* 2009). Understanding sleeping brain function throughout normal aging will provide an essential base from which to explore pathological patterns of brain aging.

The aim of this study was to characterize topographic changes in sleep EEG power across normal aging. We drew upon a pool of healthy adults aged 18-65 years old who had previously completed baseline studies in our laboratory using high density EEG.

METHODS

Participants and Study Design

This study analyzed data collected from 92 adults aged 18-65 years (59 women) who had participated as healthy controls in protocols at the University of Wisconsin-Madison sleep laboratory (Supplementary Figures 1 & 2). Individuals were included in the analysis if they had undergone polysomnography (PSG) with hdEEG, without behavioral or pharmacological interventions. Participants were included only if they were free from neurological, major medical and sleep disorders including sleep disordered breathing (Apnea Hypopnea Index < 5/hr) and sleep related movement disorders (Periodic Limb Movements < 8/hr). Participants were excluded if they were taking drugs known to affect sleep (assessed by self-report). 54 participants (34 women) were drawn from a study of sleep and meditation, for which individuals aged 25-65 years were recruited through newspaper advertisements, email and distribution of recruitment flyers to meditation and wellness centers.(Ferrarelli *et al.* 2013) Only participants

with no meditation experience were included in this analysis. 38 participants (22 women) were drawn from a study of normal sleeping brain activity(Riedner *et al.* 2007; Murphy *et al.* 2009; Langheim *et al.* 2011), for which healthy adults were recruited through newspaper advertisements and word of mouth. Some participants were allowed to sleep until they awoke naturally while others were awakened at 6:30am. Therefore, to ensure that functionally similar sleep periods were examined across all individuals, participants were only included if they had experienced at least 3 sleep cycles during their PSG, with < 30% wake from sleep onset to the end of the third sleep cycle. For all participants, only the first 3 sleep cycles were analyzed. All participants were right-handed, defined by the Edinburgh Handedness Scale.(Oldfield 1971)

Each participant's medical and psychiatric histories were collected through an initial phone screening, followed by a thorough in-person screening involving questionnaires assessing general medical history, anxiety and depression, and a modified version of the Sleep Disorders Questionnaire(Douglass *et al.* 1994) with 16 questions assessing symptoms of sleep apnea and periodic limb movement disorder. All participants were instructed to maintain regular sleep-wake schedules during the week prior to the study, which was confirmed with sleep diaries and wrist-worn actigraphy (Actiwatch, Mini-Mitter, Bend, OR), and to refrain from consuming caffeine and alcohol on the day of the study. Participants arrived at the laboratory between 7 and 9 pm, were set up with sensors for the sleep study (approximately 45 minutes), then went to bed within one hour of their usual bed time. They were allowed to sleep undisturbed until 6:30 am or until they awoke naturally. All data were drawn from baseline study visits; no pharmacological or behavioral interventions were performed. All participants provided informed consent, and protocols were approved by the Institutional Review Board of the University of Wisconsin-Madison.

Polysomnography

Sleep was evaluated in all participants with standard PSG monitoring including electrooculogram (EOG), sub-mental electromyogram (EMG), electrocardiogram (ECG), bilateral tibial EMG, respiratory inductance plethysmography, pulse oximetry and a position sensor using a customized Alice 5 System (Philips Respironics, Murrysville, PA). Simultaneously, high density electroencephalography (hdEEG) was recorded from 256 channels with vertex referencing using NetStation software (Electrical Geodesics Inc., Eugene, OR). Sleep was scored by a registered sleep technologist according to AASM scoring guidelines (Iber *et al.* 2007) using Alice® Sleepware (Philips Respironics, Murrysville, PA) and then reviewed by a Sleep Medicine physician certified by the American Board of Medical Specialties (RMB). Sleep staging was performed using 6 hdEEG channels located at approximate 10-20 locations (F3, F4, C3, C4, O1, O2), re-referenced to the mastoids.

HdEEG Recordings

HdEEG signals were sampled at 500 Hz and referenced to the vertex, using a NetAmps 300 amplifier and NetStation software (Electrical Geodesics Inc., Eugene, OR). In NetStation a first-order high-pass filter (0.1 Hz) was applied to mimic common hardware analog filters and to eliminate low frequency drift. Data were filtered in MATLAB (The MathWorks Inc., Natick, MA) using first order high pass (0.1 Hz) to remove low frequency artifact associated with sweating, followed by a band-pass filter (Kaiser type, 1-50 Hz). The data were then average-referenced to the mean voltage across all good channels.

Analyses were performed on the first 3 sleep cycles only, to maximize equivalence between participants. Spectral analysis of NREM sleep was performed for each channel in six-second epochs (Welch's averaged modified periodogram with a Hamming window). A semi-automatic artifact rejection was conducted to remove six-second sleep epochs that exceeded thresholds for individual channels based on the mean power in either low (1-4 Hz) or high (20-30 Hz)

frequency bands. EEG channels in which artifacts affected most of the recording were subsequently removed. Average spectral density across NREM sleep was computed in six standard frequency ranges from the literature and our own studies:(Finelli, Borbély and Achermann 2001; Andrillon *et al.* 2011; Murphy *et al.* 2011) delta (1-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), sigma (12-15 Hz), beta (15-25 Hz) and low gamma (25-40 Hz). The frequency cutoffs used to distinguish between slow and fast spindles vary throughout the literature (Ferrarelli *et al.* 2010; Andrillon *et al.* 2011; Martin *et al.* 2013). Therefore to explore differences between fast and slow spindle frequencies without selecting arbitrary cutoffs, spectral density was computed in 1 Hz bins from 9 to 16 Hz.

Statistical Analysis

Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity and homoscedasticity. The relationship between age and sleep architecture across the first 3 sleep cycles was assessed using Pearson product-moment correlation coefficient. All-night sleep architecture is not reported, because the protocols were not designed to assess this. For comparison of spectral data and for initial topographic visualization, subjects were divided into 5 age groups: 18-24, 25-34, 35-44, 45-54 and 55-64 years. Each polysomnographic variable was analyzed separately in a two-way ANOVA with factors age group and gender. Significant main effects were assessed with post-hoc Tukey tests, with $p<.05$ considered significant. Spectral power density in 1/6 Hz width bins was compared between age groups with a one-way ANOVA with factor age group. Significant comparisons ($p<.05$) were followed up with post-hoc Scheffe's tests of group differences.

For each age group topographic maps of average absolute spectral power density (μV^2) were calculated for standard frequency bands for all channels, averaged across the NREM periods of the first 3 sleep cycles. To account for inter-individual variability, normalized maps were also generated by computing the z-score across all good channels for each subject. The correlation

of age with power density was calculated for each electrode and Pearson's correlation coefficient was displayed on topographic maps. Absolute power was log transformed before the correlation with age was computed, to produce normally distributed residuals. To control for multiple comparisons, significant correlations were determined by statistical non-parametric mapping (SnPM) using a single threshold test (Smith and Nichols 2009). To visualize the relationship, the linear correlation was plotted at Fz in each frequency band. To increase the signal-to-noise ratio, statistical analyses of topographies were restricted to channels overlaying the scalp (specifically, 173 channels falling within a plotting radius of 0.57 specified in the topoplot function of the EEGLAB plug-in for MATLAB), with channels on the neck or the face excluded. All statistical procedures were performed using MATLAB (The MathWorks Inc., Natick, MA). To confirm regional differences of the age effect, a mixed ANOVA was conducted with SPSS 22, with age as the between-subjects factor and region as the within subjects factor (2 levels: frontal and left central). Frontal slow wave power was defined as the mean power in a cluster of electrodes encircling Fz. Frontal sigma power, left central slow wave and left central sigma power were defined as the mean power in the electrodes that showed a significant correlation of age with normalized power.

RESULTS

Participant Characteristics and Sleep Architecture

92 adults (59 women) met the selection criteria and were included in all analyses. The proportion of women was greater with increasing age (Supplementary Figure 1). Polysomnographic characteristics (averaged across the first 3 sleep cycles only) are summarized in Table 1. Increasing age was associated with less deep sleep, evidenced by higher percentages of wake after sleep onset and stages N1 and N2, and lower percentage of stage N3. There was no relationship between age and total sleep time or percent REM sleep

during the first 3 sleep cycles. There was no significant interaction effect of sex on these relationships.

Table 1 – Correlation Between Age and Sleep Architecture

	mean (SD)	r	p
TST (min)	271.7 (55.8)	-.18	n.s.
WASO (%)	7.9 (6.9)	.48	<.0001
N1 (%)	6.9 (4.2)	.40	<.001
N2 (%)	68.2 (11.5)	.41	<.001
N3 (%)	15.8 (11.1)	-.38	<.0001
REM (%)	19.7 (6.4)	-.16	n.s.

N=92. r, Pearson product-moment correlation coefficient (2-tailed); p, significance; n.s., non-significant. TST, Total Sleep Time; WASO, Wake After Sleep Onset (% of Time in Bed), N1/2/3/REM(%), NREM stage 1/2/3/REM (% of TST). All variables calculated for the analysis period (first 3 sleep cycles).

Spectral high density EEG Analysis

Power spectra, averaged across all channels, are plotted in Figure 1. All age groups showed the classical pattern of NREM spectral activity, with greatest power in the slow wave frequency band (1-4.5 Hz) and a second peak in the sigma band (12-15 Hz). In general, older age groups showed significantly lower power than younger age groups in the slow wave and low theta frequency ranges, and greater power than younger age groups in the high gamma range. No differences between age groups were observed in the high theta, alpha, sigma, or beta frequency ranges.

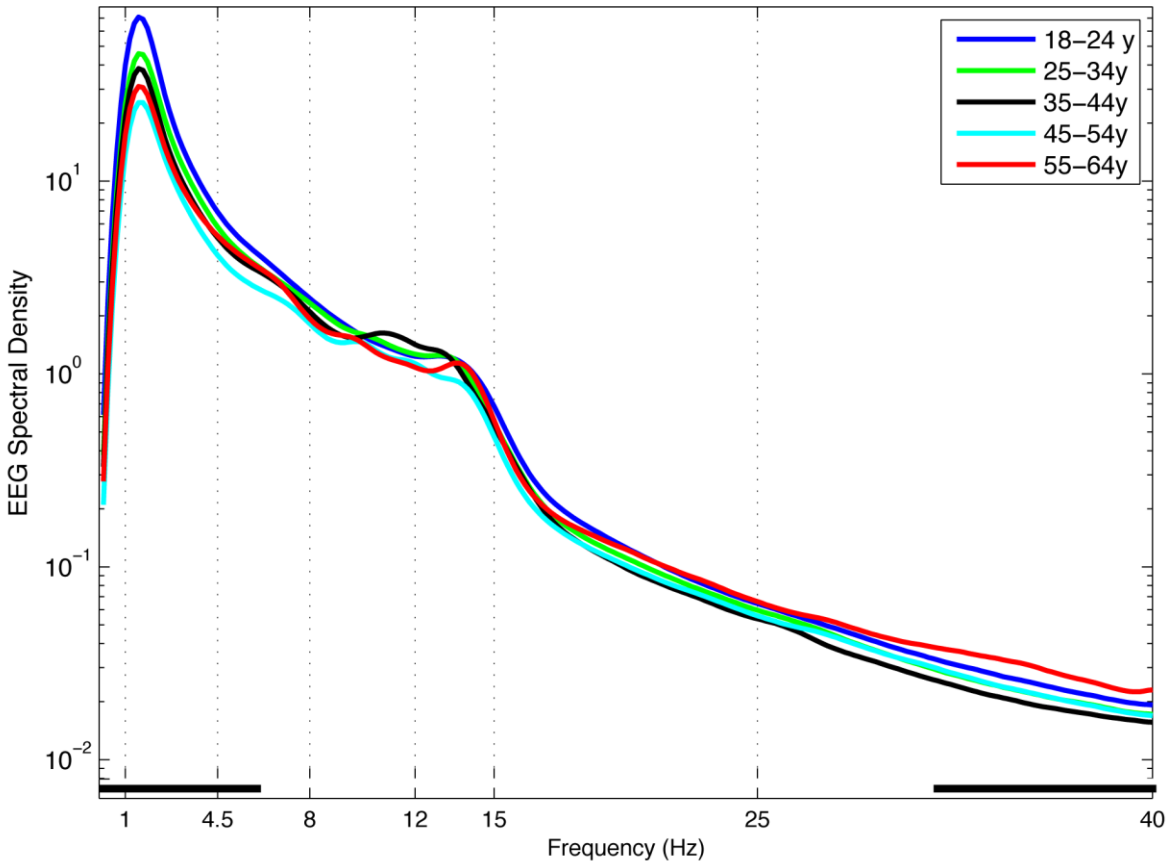


Figure 1. NREM EEG Power Spectra.

EEG power spectra (log μV^2/Hz) for NREM sleep during the first 3 sleep cycles, averaged across 173 scalp electrodes in 1/6 Hz frequency bins. Age groups plotted by decade (aged 18-25 years, dark blue; 25-35 years, green; 35-45 years, black; 45-55 years, light blue; 55-65 years, red). Classically defined frequency bands indicated by vertical dotted lines. Black squares along the x-axis indicate frequency bins in which ANOVA showed a significant effect of age group.

High density EEG topography

The topography of absolute EEG spectral power density in standard frequency bands for each age group is shown in Supplementary Figure 3. Power in the slow wave range was greatest frontally at all ages. Both frontal and posterior maxima were evident in the theta, alpha and sigma ranges. Representative scatterplots of the correlation between age and EEG power at Fz are shown in Supplementary Figure 4.

Correlation of EEG power with age.

To quantify topographic changes with age, maps were generated for the coefficient of the correlation between power and age at each electrode (Figure 2). When power was expressed as absolute values, slow wave power declined significantly with increasing age across the entire scalp, whereas power in the theta and sigma range declined significantly in a frontal cluster of electrodes adjacent to Fz. There was no significant effect of age on absolute power in the alpha, beta or gamma frequency bands.

When EEG power was normalized across the scalp, a left centro-parietal region showed significantly less age-related decline in power than the rest of the scalp. This partial preservation of left centro-parietal power was particularly prominent in the slow wave and spindle bands. The interaction of region and age was confirmed by mixed ANOVA, such that the decline of EEG power with age was greater in the frontal than left central region for the slow wave band [$F(2,90)=28.9$, $p<.0001$] and sigma band [$F(2,90)=30.8$, $p<.0001$] (Figure 3). In general, age-related power declines were greatest frontally, and this effect was statistically significant in the theta and sigma bands. The parietal shift in dominance was driven by a loss of frontal power and preservation of parietal power. It was not driven by an increase in parietal activity, because absolute power did not increase with age at any electrodes.

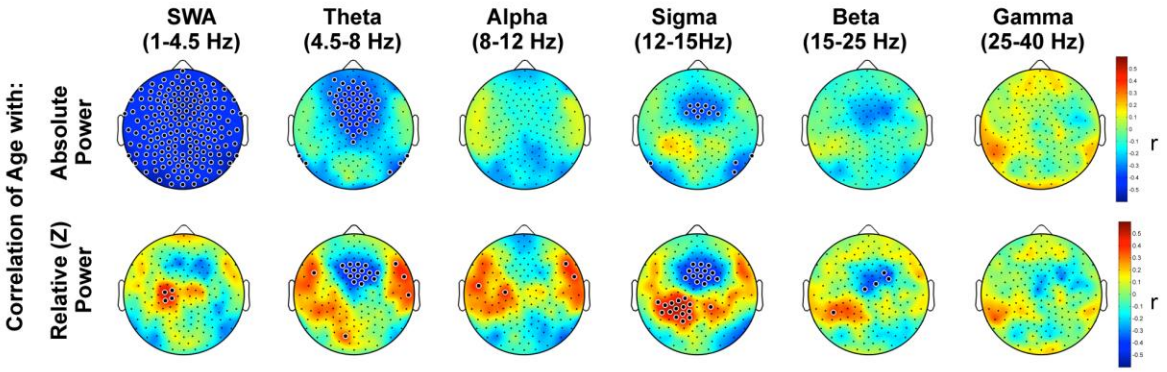


Figure 2. Topography of the correlation of age with NREM EEG power.
Topography of the correlation between age and NREM spectral density, averaged across the first three sleep cycles in standard frequency bands. Color represents the coefficient of the correlation between age and power at each electrode. In the upper panel, blue indicates a negative correlation, i.e. a decline in absolute NREM EEG power (log μV^2) with increasing age. The lower panel plots the coefficient of correlation (r) between age and power (μV^2) normalized to the scalp mean within an individual. Colors indicate that as age increases, EEG power is increasingly higher (red) or lower (blue) than the scalp mean. Large black dots indicate channels at which the correlation of age and EEG power was significant, accounting for multiple comparisons with statistical nonparametric mapping.

When spindle frequencies were examined in 1 Hz bins (Figure 4), absolute power in faster spindle frequencies (13-16 Hz) declined significantly with increasing age in a frontal cluster adjacent to Fz whereas absolute power of slower spindle frequencies (9-12) showed no significant change with age. Partial preservation of relative power in a centro-parietal region was evident across the spindle band, but was particularly prominent in the 12-15 Hz range.

In all frequency bands the effect of age on EEG topography was similar across sleep cycles, with larger clusters of electrodes showing statistical significance in earlier sleep cycles. Therefore the findings are robust, not driven by one sleep cycle (data not shown). We found no significant effect of sex on the relationship between EEG topography and age (Supplementary material).

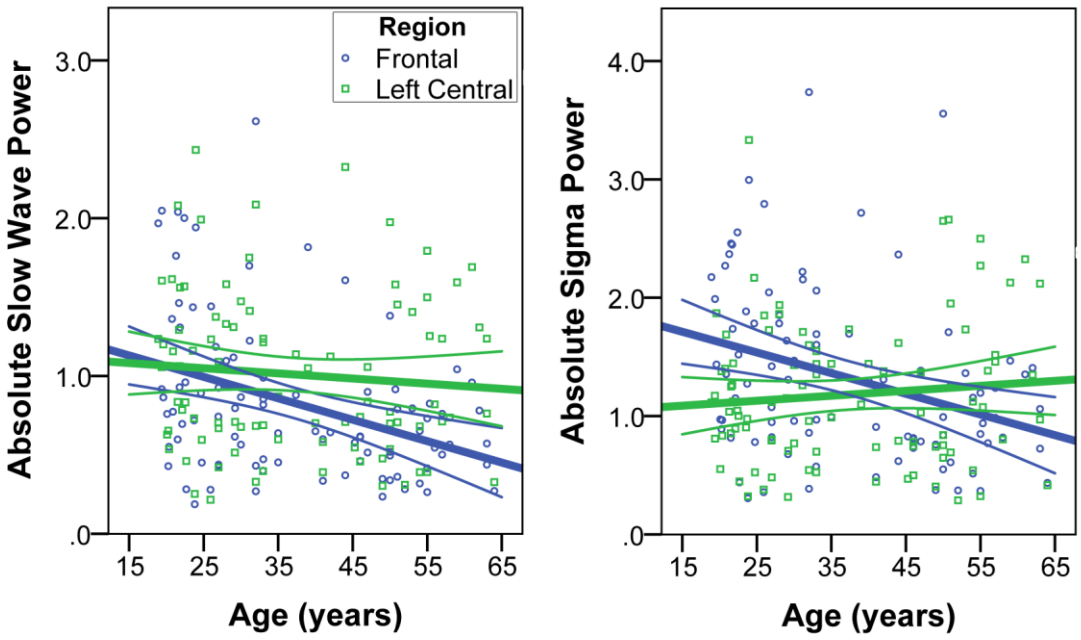


Figure 3. Interaction of age and region on EEG Power.
Correlation of age and absolute NREM EEG power (log μV^2) in the Slow Wave (left) and Sigma (right) frequency bands. In each band, power was averaged in a frontal (blue circles) and left central (green squares) region, defined by clusters of electrodes showing significant correlation of age and normalized power. Mixed ANOVA revealed a significant region x age interaction, such that the age-related decline of EEG power was greater in the frontal than left central region for slow wave and sigma bands.

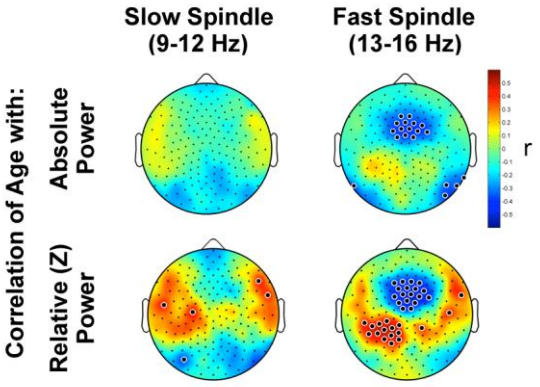


Figure 4. Topography of the correlation of age with NREM EEG spectral density in spindle frequencies.
Topography of the correlation between age and NREM EEG spectral density (log μV^2), in 1 Hz bins of spindle frequencies, averaged across the first three sleep cycles. Color represents the coefficient of the correlation between age and power at each electrode. Small black dots indicate electrode locations. Large black dots indicate electrodes at which the correlation of age and EEG power was significant, accounting for multiple comparisons with statistical nonparametric mapping.

DISCUSSION

This study examined age-related changes in the topography of sleep EEG power across an age range of 18-65 years. The study design had three key strengths. First, the high spatial resolution of hdEEG used in this study allowed identification of regional effects. Second, age was treated as a continuous variable, rather than comparing groups from opposite ends of the age spectrum. Finally, this study included middle-aged adults, a critical period for brain health that has been largely neglected in studies of sleep and aging.

The effect of age on EEG power varied considerably by region and across frequency bands. We focus our discussion primarily on the slow wave and spindle frequency range, as these waveforms are known to support cognitive processes that change substantially across the lifespan, such as learning and memory.(Mander *et al.* 2013a, 2013b)

Scalp-Wide Decline in Absolute Slow Wave Power

With increasing age, slow wave (SW) power declined significantly across the entire scalp. Prior studies reported a frontal dominance of age effects on slow waves,(Landolt and Borbély 2001; Münch *et al.* 2004) based on recordings from only 3 or 4 electrodes along the antero-posterior midline (Fz, Cz, Pz, Oz). The disparity could be due to the EEG referencing techniques used. We used an average reference because this technique has better performance than other commonly used schemes (Nunez 2010; Liu *et al.* 2015). Prior reports referenced to the mastoids, which can distort the EEG signal by removing more physiologic information closer to the mastoids (i.e. central and parietal regions) than in more distant regions (i.e. frontal) (Qin, Xu and Yao 2010). This could explain why frontal effects were more prominent in the prior reports. The scalp-wide topography of SWA decline we observed could be due to high proportions of women in the older age groups in our sample; an age-related decrement in SW incidence was found to be greater frontally in men, but was equally distributed along the antero-posterior axis in women(Carrier *et al.* 2011). We found that the effect of age on EEG spectral power did not differ by sex, consistent with some (Carrier *et al.* 2001) but not all (Ehlers and Kupfer 1997; Carrier *et al.* 2011) previous reports.

Declining SW power in older age is likely driven by anatomical changes. Aging is associated with widespread atrophy of grey matter (Giorgio *et al.* 2010; Raz *et al.* 2010), as well as a regional thinning of grey matter that mediates an age-related reduction in SW amplitude and density (Dubé *et al.* 2015). In addition, individual differences in SW power correlate with white matter volume and microstructure(Buchmann *et al.* 2011a; Piantoni *et al.* 2013). Slow waves are primarily maintained and synchronized by cortico-cortical connections(Murphy *et al.* 2009), and more synchronous firing leads to greater summation of electrical potentials, observable as greater SW amplitude and power(Vyazovskiy *et al.* 2009). Given that DTI measures of white matter structure begin to decline after age 20 years(Lebel *et al.* 2012), atrophy or degradation of

white matter could diminish synchronization, thereby contributing to SW power reduction scalp-wide.

In addition to changes in gross brain structure, the loss of SW power with age may also be driven by synaptic changes. It has been repeatedly suggested that changes in SW power across childhood could be driven by synaptic proliferation and subsequent pruning, motivated by the observation that SW power, synaptic density and brain metabolism follow parallel inverted-U shaped curves up to late adolescence (Feinberg *et al.* 1990; Murphy *et al.* 2009; Feinberg and Campbell 2012; Piantoni *et al.* 2013). It follows that the further decline in SW power across adulthood could be due to continued synaptic loss. However, a recent study testing this hypothesis in mice failed to find an association between SW power and synaptic density across adolescence(de Vivo *et al.* 2014). Instead, the loss of SW power might result from a more subtle reorganization of cortical circuits. For example, some classes of synapses are more vulnerable to aging than others; thin spines accounted for the majority of spine loss in prefrontal cortex whereas mushroom and stubby spines remained relatively stable with aging in rhesus monkeys(Dumitriu *et al.* 2010). Given that spine morphology affects the strength and dynamics of synaptic transmission, spine changes across aging could result in altered circuit synchronization and lowered SW power.

Slow waves are thought to reflect and participate in plastic processes during sleep(Tononi and Cirelli 2012). It has already been shown that in a frontal region, age-related declines in SW power correlate with decrements in memory performance(Mander *et al.* 2013a). Our findings of a scalp-wide decline in SW power suggest that aging may be associated with impairments in slow-wave mediated plasticity across the entire cortex.

Frontal Declines in Absolute Theta and Sigma Power

In contrast to the scalp-wide decline in SW power, the age-related decline in theta and sigma

power was focused on a frontal region, with no significant declines posteriorly. This pattern is consistent with a previous report using only three mid-line derivations(Landolt and Borbély 2001). Grey matter volume correlates with individual differences in maximal theta and sigma power(Buchmann *et al.* 2011a); therefore the frontal pattern of theta and sigma decline observed here could be due to atrophy of frontal grey matter known to accompany aging(Giorgio *et al.* 2010; Raz *et al.* 2010). Oscillations in the theta range (4-7 Hz) during wakefulness promote synaptic plasticity and are linked to memory encoding(Klimesch *et al.* 1996; Sederberg *et al.* 2003; Osipova *et al.* 2006), and there is evidence that frontal theta plays a similar role during REM and NREM sleep(Marzano *et al.* 2011; Westerberg *et al.* 2012). Thus disruptions of theta oscillations during sleep may contribute to age-related declines in memory performance. The sigma band corresponds to the frequency of spindles, which are discussed in further detail below.

Preservation of EEG Power in a Left Centro-Parietal Partial Region

Age-related declines in EEG power were less pronounced in a left centro-parietal region. This asymmetry has not been previously described, because previous studies of topography and aging restricted analyses to midline derivations or to one hemisphere. The relative preservation was in the dominant hemisphere (all participants were right-handed). With increasing age, loss of dexterity is more pronounced in the non-dominant hand(Amirjani *et al.* 2007), and atrophy of grey matter is more pronounced in the sensorimotor hand area of the non-dominant hemisphere compared to the dominant hemisphere(Bonilha *et al.* 2009). Therefore the preservation of EEG power over left centroparietal cortex could be due to a larger neural population contributing to oscillations in that region. Alternatively, the preservation of power may reflect sleep-mediated plastic processes. Regional SW power reflects prior use, for example sleep SW power is increased in parietal cortex following a motor training task(Huber *et al.* 2004; Landsness *et al.* 2011). Older adults experiencing reduced dexterity in the non-dominant hand may compensate

by using the dominant hand more frequently and by learning new motor patterns adapted to their abilities. This motor learning could be reflected in increased parietal SW power.

Age-Related Decline of Fast Frontal Spindle Band Power, But Not Centroparietal Spindle Band Power

Spindles fall into two distinct classes: fast and slow. Although their precise origins and functions are uncertain, the two classes have different topographies (Ferrarelli *et al.* 2010; Andrillon *et al.* 2011; Martin *et al.* 2013), are differently affected by pharmacological agents (Ayoub *et al.* 2013) and clinical disorders (Ferrarelli *et al.* 2013; Nishida, Nakashima and Nishikawa 2014; Plante *et al.*) and make distinct contributions to cognition (Mölle *et al.* 2011; Chatburn *et al.* 2013). The frequency cutoffs used to define each class vary throughout the literature; therefore we examined activity in 1Hz bins. We observed the predicted slow-frontal, fast-central topographic distinction in all age groups. Our finding of an age-related decrease of frontal power in faster (13-15 Hz) but not slower (9-12 Hz) spindle frequencies confirms that slow and fast spindles are distinct phenomena that are differently affected by age.

The age-related decline of power in fast spindle frequencies was confined to a frontal region, where fast spindles are usually less prominent (Andrillon *et al.* 2011). Others have reported age-related declines in spindle band power but the few reports of topographic changes have been mixed. Landolt and Borbely(Landolt and Borbély 2001), found a posterior shift in slow (10.25-12Hz) but not fast spindle band power in old compared to young adults. Using spindle detection algorithms at 5 midline derivations, Martin et al. (Martin *et al.* 2013) found that older adults showed lower spindle density (spindles/min) and amplitude in frontal derivations, whereas an age-related reduction in duration was strongest in posterior derivations. They did not categorize spindles as fast and slow, but did report that spindle frequency was lower in younger adults in posterior derivations during later sleep cycles only. Based on these reports, the frontal loss of fast spindle band power that we observed could be due to reduced spindle amplitude and

density, and is unlikely to be due to changes in spindle frequency. Fast spindles are more strongly tied to slow wave upstates than are slow spindles(Andrillon *et al.* 2011); their loss may be via a shared mechanism with the decline in frontal slow waves.

The precise function of spindles is uncertain but there is evidence for a role in learning and memory and as an attentional 'gate' protecting sleep by regulating the salience of external stimuli (Steriade 2003; Walker and Stickgold 2006). The functional significance of each spindle class and the implications of selective loss of frontal fast spindle band power are unclear. When a prefrontal region was examined a priori, fewer prefrontal fast spindles (13.5-15 Hz) statistically mediated poorer memory function in older (71.9 ± 6.7 years) compared to younger (20.5 ± 2.1 years) adults (Mander *et al.* 2013b). Spindles are generated in the reticular formation of the thalamus and conveyed to the cortex by thalamo-cortical projections (Steriade 2003). Thus age-related alterations in spindle band power could arise from changes in the thalamus, the cortex or thalamo-cortical projections. Anterior and medial thalamic aspects of the thalamus and their projections to frontal cortex show signs of atrophy with increasing age, while the volume of thalamic projections to parietal, temporal and occipital cortex show no significant relationship with age (Giorgio *et al.* 2010; Hughes *et al.* 2012). Age-related deterioration of anterior thalamic nuclei and their projections could therefore impair transmission of fast spindles from the thalamic nuclei to frontal cortex. However the source generators and transmitting fibers of distinct spindle classes are still uncertain and it is unclear why the slow spindle band, which is frontally dominant, was not affected by age.

Limitations and Strengths of This Study

Limitations of this study include the cross-sectional design and the lack of ad-libitum sleep, which precluded analyses of sleep architecture and time-of-night effects. Data was only analyzed from one night for each participant, however the scalp topography of sleep EEG shows a consistent pattern across nights within individuals (Finelli, Achermann and Borbély

2001; Tinguely *et al.* 2006). Hormonal and menopausal status of women was not assessed in our sample, which could have influenced findings; for example, activity in alpha, sigma and beta frequencies varies across the menstrual cycle (Baker *et al.* 2012) and menopause is associated with elevated beta activity (Campbell *et al.* 2011). We found, however, that the effect of age on EEG spectral power did not differ by sex. This is consistent with previous reports that although EEG spectral density differs between men and women, the effect of age does not vary by sex (Ehlers and Kupfer 1997; Carrier *et al.* 2001).

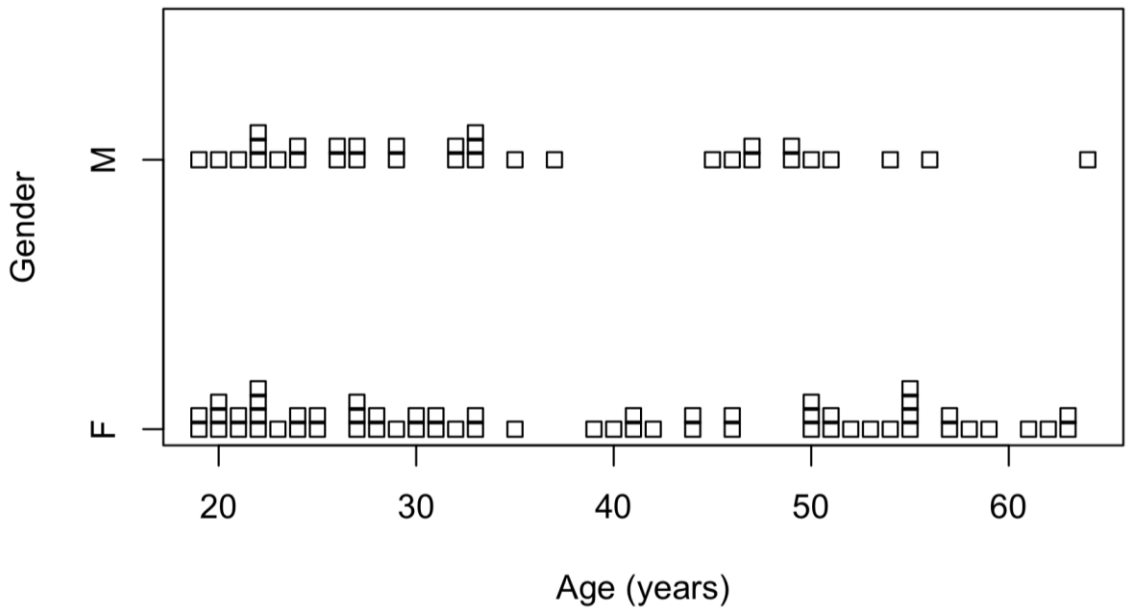
A key strength of the current study was that it examined brain change across aging by treating age as a continuous variable, whereas previous studies of EEG topography have compared age groups at opposite ends of the age spectrum. Aging is a progressive, rather than step-wise process therefore the treatment of age as a continuous variable is a more appropriate method of evaluating the brain changes associated with aging. It will be important to confirm the age-related changes observed in this cross-sectional dataset with longitudinal studies within individuals.

Conclusion

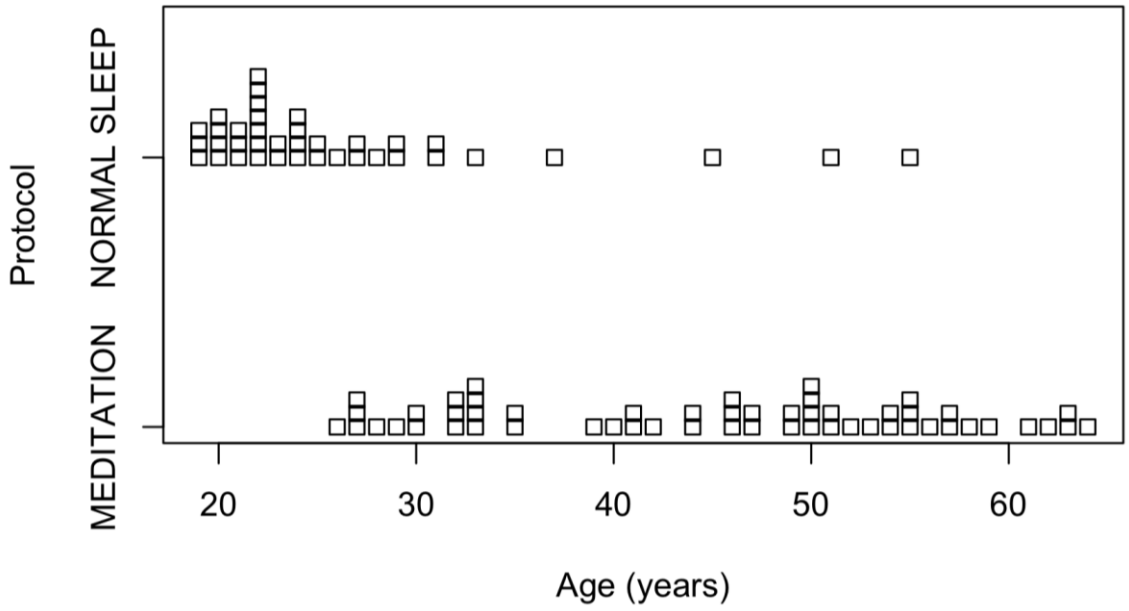
This study provides normative data on the effect of age on sleep EEG topography with high spatial resolution. These results are relevant for interpreting sleep EEG changes in disorders that present later in life. For example, the aging-related pattern of scalp-wide SW decline and frontal theta and sigma decline differs markedly from the posterior and parietal EEG changes we have observed in disorders for which older age is a risk factor including OSA (Jones *et al.* 2014) and insomnia (Plante *et al.*). Given the substantial effect of age on sleep EEG power and topography, it is important for future studies of sleep EEG to control for age in their statistical models and study design. Furthermore, given the asymmetric effect of age on EEG topography, future study designs should consider regional variation in their measures. The functional and anatomical correlates of age-related EEG topography changes in each frequency band also

require further investigation. Understanding normal sleeping brain activity across adulthood provides an important basis from which to explore pathological patterns of brain aging.

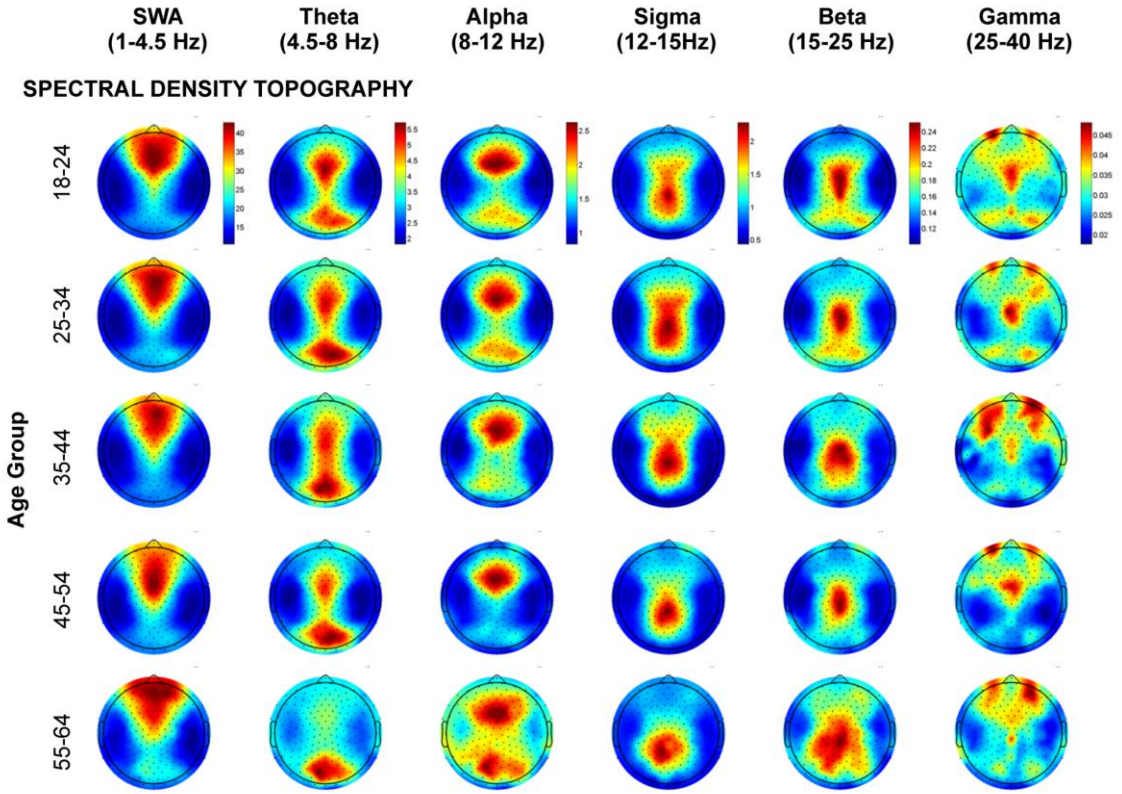
SUPPLEMENTARY MATERIAL



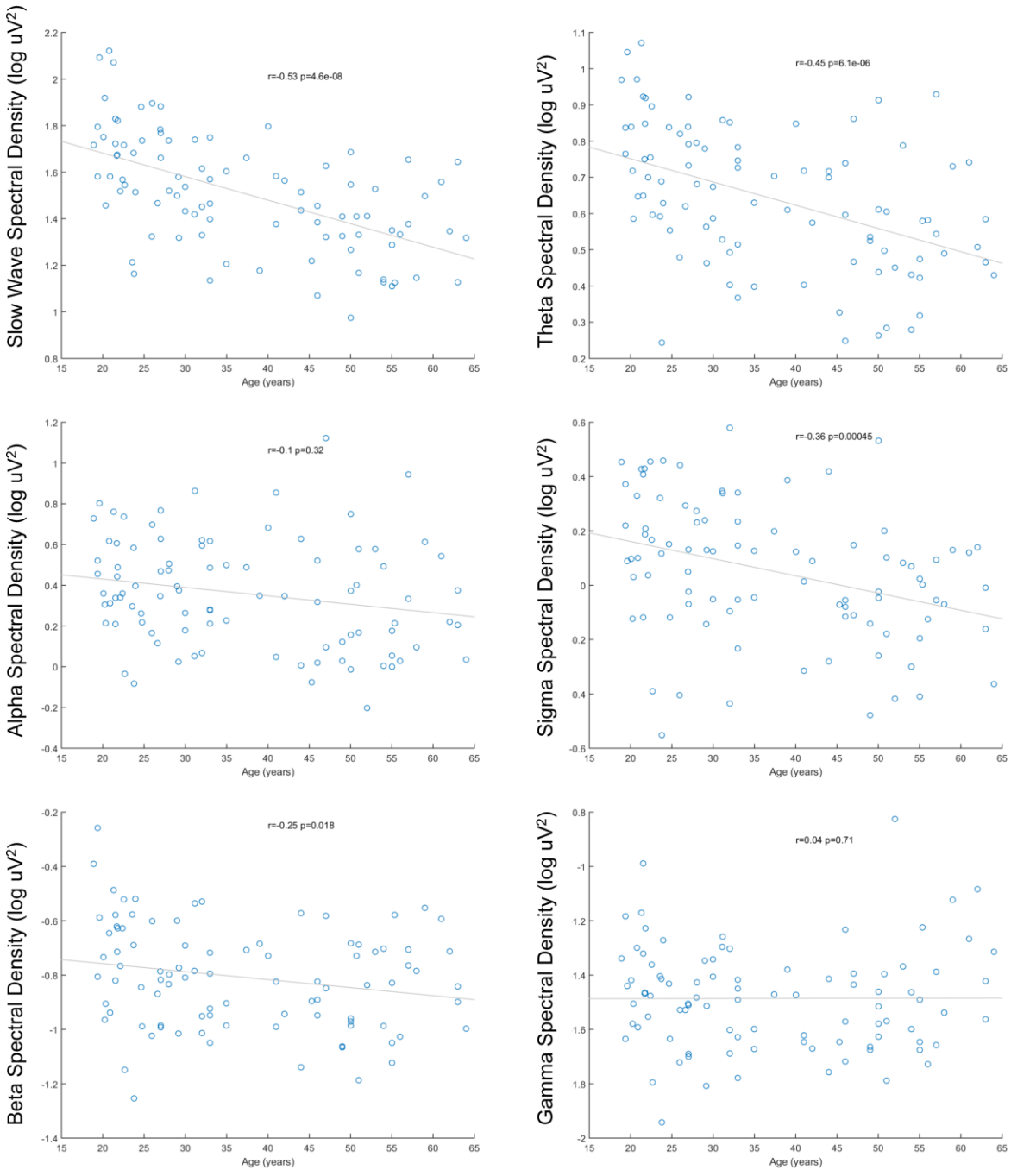
Supplementary Figure 1
Distribution of participant age, separated by gender. Males are represented by red dots; women are represented by black dots.



Supplementary Figure 2
Distribution of participant age, separated by protocol from which they were sourced.



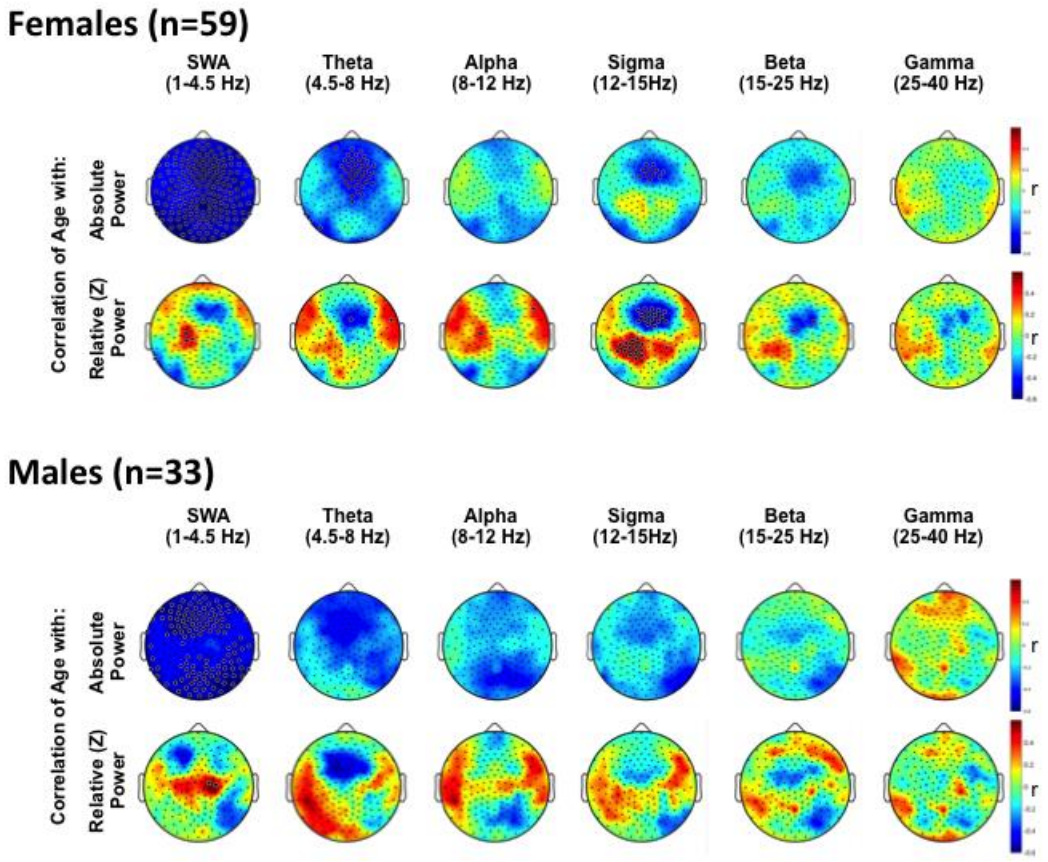
Supplementary Figure 3
Topography of absolute EEG spectral density in standard frequency bands, averaged across the first 3 sleep cycles, binned into arbitrary age groups by decade.



Supplementary Figure 4
Correlation of age (years) with NREM EEG spectral density at Fz (log μV^2), in standard

Interaction of sex with the effect of age on EEG topography.

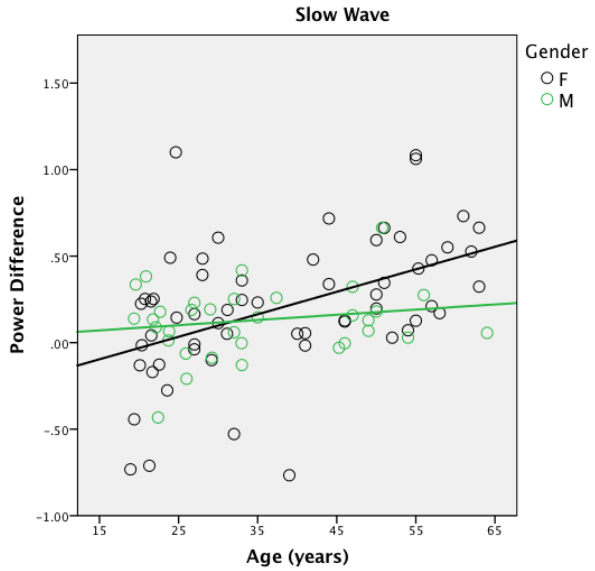
Given the lack of men aged over 50 years (n=5), these analyses should be considered exploratory only. To investigate the interaction of sex with the effect of age on EEG topography, we performed the topographic analysis separately in women and men, and observed a similar topography of the correlation of age and EEG power. The small sample of men (n=33), especially in the older age range (see supplemental figure 5) may account for the lack of statistical significance in men.



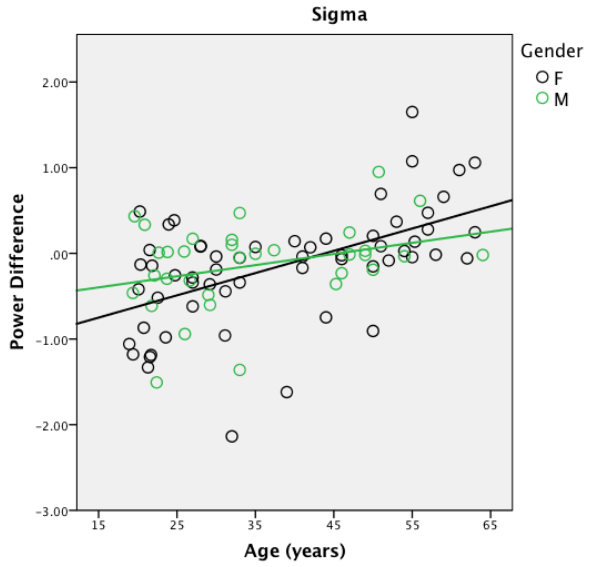
Supplementary Figure 5. Topography of the correlation of age with NREM EEG Spectral density, by sex. Topography of the correlation between age and NREM EEG spectral density, averaged across the first three sleep cycles in standard frequency bands. Top panel shows females, bottom panel shows males. Color represents the coefficient of the correlation between age and power at each electrode. Small black dots indicate electrode locations. Large black dots indicate electrodes at which the correlation of age and EEG power was significant, accounting for multiple comparisons with statistical nonparametric mapping.

To reduce the number of statistical tests, we restricted our analyses to regional difference scores in the slow wave and sigma bands. As described in the body of the manuscript, we found that the effect of age on slow wave and sigma power was greater frontally than centrally, such that the decline of EEG power with age was greater in the frontal than left central region (Figure 3 of the main manuscript). After averaging EEG power in frontal and central clusters (defined under Methods), we subtracted frontal power from central power to create a difference score for each individual, in each band. We then regressed the difference score on age, sex and age*sex.

In the slow wave band there was a significant effect of age, no significant effect of sex, and a marginally significant interaction effect of sex and age on the difference between frontal and central power (b=.01, F(3,88)=8.1, p=.047) (Supplementary Figure 6). Testing the simple effects revealed that the difference between frontal and central power increased significantly with age in females (b=.013, F(1,57)=16.7, p<.001) but not males (b=.003, F(1,31)=.93, p=.34). Sex did not significantly interact with the relationship between age and sigma topography (b=.012, F(3,88)=10.1, p=.18) (Supplementary Figure 7).



Supplementary Figure 6. Effect of age and sex on the difference between frontal and central SW power.



Supplementary Figure 7. Effect of age and sex on the difference between frontal and central sigma power.

CHAPTER 5

Regional Deficit in Sleeping Brain Activity Associated With Tau and Amyloid Pathology in Cognitively Healthy Middle-Aged Adults

Regional Deficit In Sleeping Brain Activity Associated With Tau And Amyloid Pathology In Cognitively Healthy Middle-Aged Adults

Prepared following guidelines for the journal BRAIN

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ABSTRACT

Background: Slow wave sleep is critical for memory function and brain regions that support slow wave activity are among those affected earliest in the AD process. Recent work suggests that sleep and AD pathology may have a reciprocal relationship for reasons not fully understood. The goal of this study was to assess the relationship between local deficits in slow wave sleep and accumulation of AD neuropathology among cognitively asymptomatic middle-aged adults. We hypothesized that local deficits in slow wave power would be associated with greater AD neuropathology.

Methods: 21 participants (mean age 60.05 years, 95% female, 71% with parental history of AD) were recruited from the Wisconsin Alzheimer's Disease Research Center. Only those who were cognitively asymptomatic and did not have sleep apnea were included. AD neuropathology was assessed using cerebrospinal fluid (CSF) that was collected via lumbar puncture and assayed for Aβ42 and T-Tau.

Sleep was evaluated with standard polysomnography (PSG) and with high density electroencephalography (EEG; 256 electrodes). After manual artifact removal (including EEG arousals), spectral analysis of Non-Rapid Eye Movement sleep was performed for all 256 channels (fast Fourier transform routine, Hanning window, 6s epochs). Topographic maps were then generated of the correlation between power in the slow wave range (1-4.5 Hz) and Aβ42, Aβ42/Aβ40, T-tau and T-tau/Aβ42. Significant regional correlations were determined by statistical non-parametric mapping. Power was then extracted from the significant clusters, for further analyses, controlling for age and time between CSF and PSG collection.

Results: Lower Aβ42 and T-tau and T-tau/Aβ42 were associated with lower slow wave power in frontal regions, and increased power in a centro-parietal region.

Conclusions: Local alterations in sleep slow wave activity in brain regions affected early in AD are associated with CSF biomarker evidence of AD neuropathology. This relationship, in the absence of cognitive decline, suggests that altered slow wave activity may contribute to early AD pathogenesis, and slow wave topography may itself represent a very early biomarker of AD.

INTRODUCTION

Slow wave sleep is critical for memory function (Walker and Stickgold 2006) and brain regions that support slow wave activity are among those affected earliest in the Alzheimer's disease (AD) process (Thal *et al.* 2002; Murphy *et al.* 2009; Holth, Patel and Holtzman 2017). Recent work suggests that sleep and AD pathology may have a reciprocal relationship for reasons not fully understood.

Several lines of evidence suggest that sleeping brain activity, measured by electroencephalography (EEG), may be altered by AD pathology. EEG arises from synchronous firing of pyramidal neurons, and depends on both structure and synaptic function of the brain (Steriade 2003; Buchmann *et al.* 2011a; Piantoni *et al.* 2013). The hallmark brain pathologies of AD are the accumulation of amyloid (Aβ) in plaques and tau in neurofibrillary tangles, beginning decades before cognitive symptoms (Jack *et al.* 2013). Amyloid plaques are associated with impaired cortical glucose metabolism (Johnson *et al.* 2014) and cognitive decline in AD is highly correlated with synaptic loss (Corder *et al.* 1993; Farrer *et al.* 1997) and neuronal dysfunction (Haxby and Rapoport 1986). Amyloid plaques alter neuronal firing rates and impair the ability of cortical neurons to fire synchronously (Stern *et al.* 2004; Busche *et al.* 2008). Aβ and tau interactively impair synaptic and network function (Palop and Mucke 2010; Roberson *et al.* 2011). Slow wave activity is dependent on healthy structural connectivity (Murphy *et al.* 2009; Piantoni *et al.* 2013), and white matter tracts appear compromised early in the AD process (Bendlin *et al.* 2010a, 2012; Adluru *et al.* 2014; Hoy *et al.* 2017). However little is known of whether sleep slow wave activity is altered in the presence of AD neuropathology.

The goal of this study was to assess the relationship between regional deficits in slow wave sleep and accumulation of AD neuropathology among cognitively asymptomatic middle-aged adults. We hypothesized that local deficits in slow wave power would be associated with greater preclinical AD neuropathology.

METHODS

Participants and Study Design

Participants were recruited from an ongoing longitudinal cohort of 300+ cognitively asymptomatic individuals who are part of the Wisconsin Alzheimer's Disease Research Center clinical core. Participants were recruited into the cohort from the community via advertisements and word of mouth. Participants underwent extensive cognitive testing, medical history assessment and CSF collection, bi-annually at the University of Wisconsin-Madison. Polysomnography was performed as part of a separate sub-study. Participants were recruited to polysomnography if they did not currently use therapy for sleep disordered breathing (e.g. CPAP), and did not have any major neurological, medical or psychiatric illness. All participants were included in the present analysis who were cognitively normal, had available assayed CSF samples, had completed polysomnography and did not have sleep apnea (n=21). When CSF was available from multiple time-points, the data from the CSF sample collected closest to the polysomnography was used. All participants provided informed consent, and protocols were approved by the Institutional Review Board of the University of Wisconsin-Madison.

Polysomnography

Sleep was evaluated in all participants using standard polysomnography (PSG) including electrooculogram (EOG), sub-mental electromyogram (EMG), electrocardiogram (ECG), bilateral tibial EMG, respiratory inductance plethysmography, pulse oximetry and a position sensor using a customized Alice 5 System (Philips Respironics, Murrysville, PA). Simultaneously, high density electroencephalography (hdEEG) was recorded from 256 channels with vertex referencing using a NetAmps 300 amplifier and NetStation software (Electrical Geodesics Inc., Eugene, OR). Sleep staging was performed using 6 hdEEG channels located at approximate 10-20 locations (F3, F4, C3, C4, O1, O2), re-referenced to the contralateral mastoid. A registered sleep technologist scored sleep, following AASM scoring

guidelines (Iber *et al.* 2007) using Alice® Sleepware (Philips Respironics, Murrysville, PA). A Sleep Specialist physician certified by the American Board of Medical Specialties reviewed the scoring and assessed the presence of sleep disorders, including sleep apnea.

EEG Spectral Analysis

All EEG processing was performed with custom built scripts in MATLAB (The MathWorks Inc, Natick, MA), as in our other recent studies (Jones *et al.* 2014; Riedner *et al.* 2016; Sprecher *et al.* 2016). EEG signals were collected at 500Hz, high-pass filtered at 0.1 Hz, then downsampled to 128 Hz before band-pass filtering (1-40Hz, two-way least squares FIR). The recording was divided into consecutive 6-second epochs, and epochs or channels that containing a majority of artefactual signal were removed through a semi-automatic process detailed previously (Riedner *et al.* 2016). EEG was collected with vertex referencing, then re-referenced to the average of all good channels. Absolute spectral power density was computed in the slow wave range (1-4.5 Hz), averaged across all NREM epochs. To account for inter-individual variability, normalized power was computed the z-score across all good channels for each subject. Following statistical topographic mapping, slow wave activity (SWA) was extracted from frontal and parietal clusters, defined for each CSF biomarker as a cluster of contiguous electrodes showing significant ($p<.05$) correlation with CSF biomarkers. SWA was averaged within each cluster.

CSF Collection and Analysis

Cerebrospinal fluid (CSF) was collected in the morning following a 12-hour fast. A Sprotte 25- or 24- gauge spinal needle was used to perform a lumbar puncture at L3/4 or L4/5. Approximate 22mL of CSF was obtained via gentle extraction into polypropylene syringes, then gently mixed to remove gradient effect and centrifuged for 10 minutes at 2000g. Supernatants were frozen in 0.5 mL aliquots in polypropylene tubes, and stored at -80°C. Samples were sent in 2 batches to the University of Gothenberg, Sweden, where they were assayed in a single round of analyses using one batch of reagents, yielding intra-assay coefficients of variation below 10% (within

batch). Assays were conducted by board-certified laboratory technicians blinded to participant clinical characteristics.

For statistical analysis, Aβ42 and t-tau were assessed alone and in ratios. Aβ42 was expressed in ratio to Aβ40 (the more abundant, non-toxic species), to assess the pathologic fragment (Aβ42) while accounting for individual differences in overall amyloid production. For this ratio, Aβ42 and Aβ40 were quantified by electrochemiluminescence using an Aβ triplex assay (MSD Human Aβ peptide Ultra-Sensitive Kit, Meso Scale Discovery, Gaithersburg, MD). T-tau was expressed in ratio to Aβ42 to reflect coincident pathology and because this ratio has superior diagnostic and prognostic power than either species expressed alone (Janelidze *et al.* 2016; Racine *et al.* 2016). For this ratio, Aβ42 and t-tau were quantified with a sandwich enzyme-linked immunosorbent assay (ELISA) (INNOTEST β-amyloid1-42 and hTAU-Ag respectively; Fujirebio Europe, Ghent, Belgium).

Statistical Analysis

The correlation of each CSF biomarker with absolute and normalized spectral power density was determined for each electrode, and the Pearson's correlation coefficient was displayed on topographic maps. To control for multiple comparisons, significant correlations were determined by statistical non-parametric mapping (SNPM) using threshold free cluster enhancement (Nichols and Holmes 2002; Mensen and Khatami 2013). To further explore the relationship between CSF and SWA, a regression model was used with cluster SWA as the independent variable and CSF as the dependent variable, covarying for CSF batch, time between CSF collection and PSG, and age at CSF collection (all covariates mean centered).

RESULTS

Table 1. Participant Characteristics

	Mean (±SD)

Demographics	
Age at PSG (y)	60.05 (±5.05)
Age at CSF Sample (y)	57.27 (±4.66)
Interval from CSF sample to PSG (y)	2.79 (±1.11)
Female (%)	95.2
APOE ε4 Positive (%)	38.1
Parental History Positive (%)	71.4
BMI (kg/m^2)	25.33 (±3.62)
Sleep	
Total Sleep Time (min)	345.88 (± 69.87)
Sleep Efficiency (%)	79.51 (±10.62)
Wake After Sleep Onset (min)	75.31 (±42.14)
Stage N1 (%)	5.93 (±4.06)
Stage N2 (%)	54.89 (±9.39)
Stage N3 (%)	20.80 (±9.68)
AHI (#/hr)	.88 (±1.16)
PLMS with Arousals (#/hr)	3.35 (±3.66)
Epworth Sleepiness Scale	6.75 (±3.05)
CSF Amyloid	
Aβ42 (TRIPLEX) (ng/L)	761.71 (±494.20)
Aβ42 (INNOTEST) (ng/L)	789.68 (±147.03)
Aβ42/Aβ40	.10 (±.01)
T-tau (ng/L)	247.89 (±109.98)
T-tau/Aβ42	.30 (±.10)

APOE ε4, ε4 allele of apolipoprotein E; BMI, Body Mass Index; MMSE, Aβ, amyloid beta; T-tau, total tau

Participant Characteristics and Sleep Architecture

Participant characteristics are summarized in Table 1. Participants were in late middle age (60.05 ±5.05 years) and cognitively normal. The sample was enriched with AD risk factors compared to the general population (71.4% parental history of AD, 38.1% *APOE* ε4 positive).

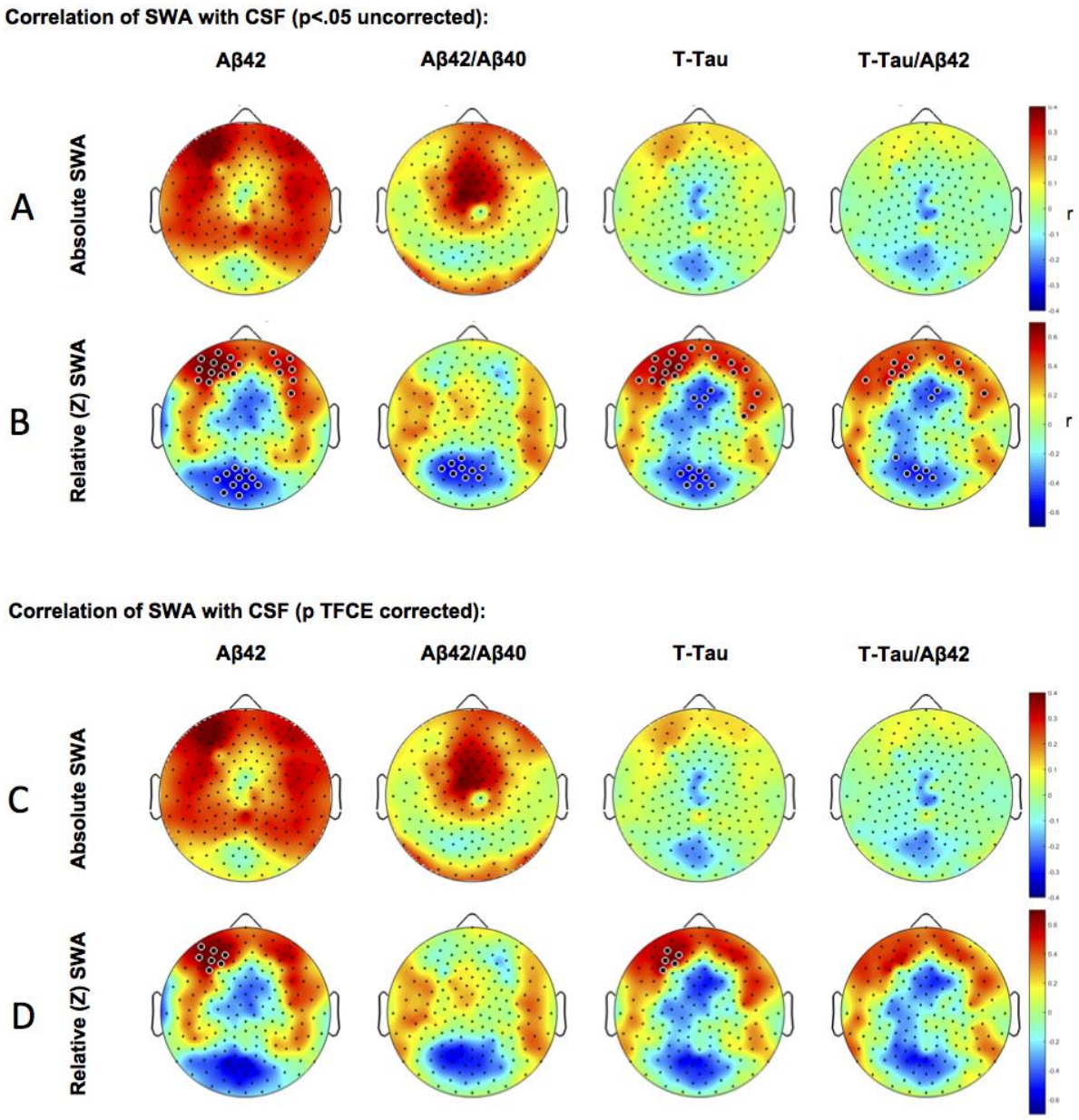

Figure 1. Topography of the correlation of SWA with CSF amyloid and tau.
Correlation between CSF biomarker concentration (μL/ml) and Slow Wave Activity (μV^2). Panels A and C: Colors represents the coefficient of the correlation between CSF and absolute SWA at each electrode, indicating that as CSF increases, absolute SWA increases (red) or decreases (blue). Panels B and D: SWA was normalized to the scalp mean within each individual. Colors indicate that as CSF increases, SWA is increasingly higher (red) or lower (blue) than the scalp mean. White outlines indicate electrodes at which the correlation of CSF and SWA was significant at p<.05 (A and B) or accounting for multiple comparisons with threshold free cluster enhancement (C and D).

Association of CSF biomarkers with Topography of Slow Wave Activity

The topography of the correlation of SWA with CSF amyloid and tau are shown in Figure 1. When SWA was expressed as absolute values, there were no significant correlations with any CSF biomarker. When SWA was normalized across the scalp for each individual, CSF Aβ42, Aβ42/Aβ40, T-tau and T-tau/Aβ42 were negatively correlated with parietal SWA. Aβ42, T-tau and T-tau/Aβ42 were positively correlated with frontal SWA. The only associations to survive TFCE correction for multiple comparisons were the positive correlation of Aβ42 and T-tau with left frontal SWA.

The results of regressions of CSF biomarkers on SWA within significant clusters are summarized in Table 2 and visualized in Figure 2. The majority of associations between SWA and CSF remained significant after controlling for covariates, apart from the association of parietal SWA with T-tau and T-tau/Aβ42.

Table 2. Results of multiple regression of CSF biomarkers on NREM Slow Wave Activity.

CSF	**SWA Cluster**	β unstandardized	β standardized	R^2	p
Aβ42	Frontal	255.280	.764	.771	**.002**
Aβ42	Parietal	-150.866	-.562	.691	**.022**
Aβ42/Aβ40	Parietal	-.011	-.391	.774	**.045**
T-tau	Parietal	-75.973	-.340	.713	.114
T-tau	Frontal	138.946	.581	.783	**.013**
T-tau/Aβ42	Frontal	.104	.432	.724	.072
T-tau/Aβ42	Parietal	-.069	-.338	.709	.112

Controlling for age at CSF sample, CSF assay batch and time between CSF sample and sleep assessment (all mean centered). β, regression coefficient; R^2, overall model fit; p, p-value; bold $p<.05$.

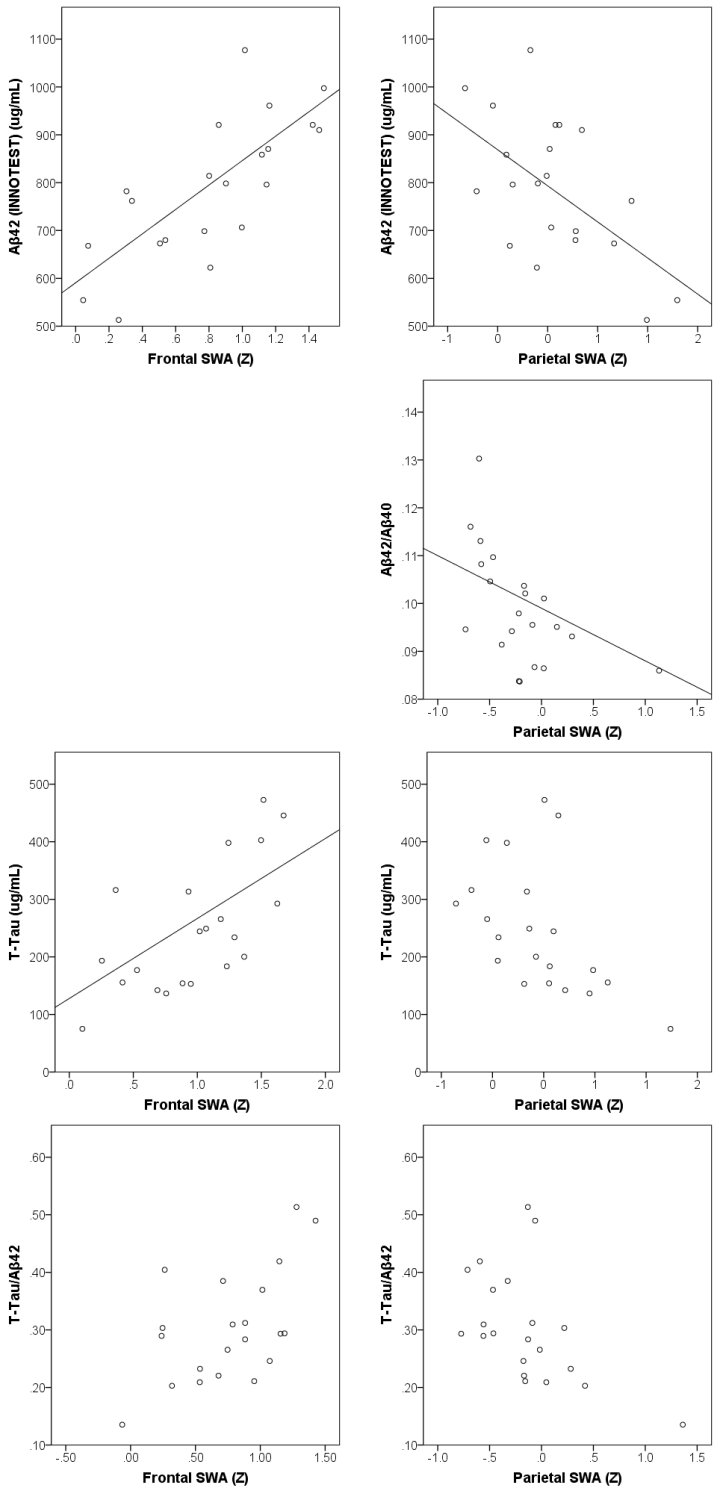

Figure 2. Association of CSF biomarkers with Frontal and Parietal SWA
Regression line is included only on plots for which the regression of CSF on SWA was significant, controlling for age at CSF sample, CSF assay batch and time between CSF sample and sleep assessment (all mean centered).

DISCUSSION

In this group of cognitively healthy adults enriched with AD risk, CSF Aβ42 and tau were positively correlated with frontal SWA and there was a trend towards a negative correlation with parietal SWA. Lower levels of CSF Aβ42 typically suggest higher amyloid plaque burden. Therefore, as we expected, the positive correlation between Aβ42 levels and frontal SWA suggests that a deficit in frontal SWA is associated with greater brain amyloid pathology. These results align with a previous report in an older group of cognitively healthy adults (mean age 75.1 years), showing that greater amyloid burden in the medial prefrontal cortex (measured with amyloid PET) was significantly associated with lower frontal SWA (measured at 10-20 sites F3,F4,F7,F8) (Mander *et al.* 2015). By contrast, Varga and colleagues (2016) found that lower SWA measured at F4 (right frontal) was significantly associated with *higher* CSF Aβ42. The reason for the inconsistency is unclear, but it could reflect differences in the populations studies and the degree to which the AD process has progressed. In the development of AD, CSF Aβ42 levels have been observed to follow an inverted-U pattern of progression, initially climbing in the earliest preclinical stages, then declining as soluble amyloid is sequestered out of the CSF and into insoluble plaques (Reiman *et al.* 2012; Maia *et al.* 2015). It is not known when the inversion point occurs, but it is thought to be a very early step, possibly decades before cognitive decline manifests (Jack *et al.* 2013). Given the small sample sizes in these studies (less than 30) it will be important to replicate these findings in a larger sample. Longitudinal studies will also be essential to better understand the progression of AD biomarkers and their shifting relationship with sleep.

Slow wave sleep is critical for the restorative function of sleep, and we have previously found that self-report of inadequate, unrefreshing sleep was associated with greater AD pathology including cortical amyloid burden and CSF markers of amyloid, tau and neuroinflammation

(Sprecher *et al.* In Press, 2015). Our new findings suggest that a loss of slow wave activity may be one mechanism linking non-restorative sleep with AD pathology.

With regard to correlations with tau, we found that less frontal SWA was associated with lower T-Tau levels. Tau is a scaffolding protein in neuronal axons. In AD, hyperphosphorylation of the tau protein leads to axonal degeneration, which in turn leads to increased tau release into the CSF and ultimately the formation of neurofibrillary tangles. Thus elevated CSF T-tau is indicative of greater pathology. The positive correlation of T-tau with frontal SWA is surprising. We expected that lower SWA would be associated with greater tau pathology because tau interferes with network activity. However, very little is known about the relationship between slow wave sleep and neurofibrillary tangles, and more research is needed in this area.

In fact, amyloid and tau appear to have different effects on sleep. Mice overexpressing amyloid develop more fragmented sleep (Roh *et al.* 2012), whereas mice overexpressing tau exhibit longer sleep bouts and fewer sleep-wake transitions, despite an overall decrease in total sleep time (Holth *et al.* 2017). On the other hand, elderly adults with a history of fragmented sleep had more neurofibrillary tangles at autopsy (Lim *et al.* 2013). Most relevant to this analysis, amyloid-overexpressing mice show decreased SWA (Zhang *et al.* 2005; Schneider *et al.* 2014a), whereas tau-overexpressing mice had increased SWA during NREM sleep (Holth *et al.* 2017). This suggests a complex interaction between sleeping brain activity, amyloid and tau, that likely evolves as AD pathology progresses. The current lack of longitudinal data makes human sleep phenotypes difficult to predict at each disease stage.

The fact that different patterns were observed in frontal versus parietal SWA in relation to CSF biomarkers is consistent with previous findings using lower resolution EEG. Mander et al (2015) found that greater cortical amyloid burden was associated with reduced slow waves frontally, but not in parietal, temporal or occipital sites. Varga et al (2016) found that that CSF Aβ42 was

significantly associated with SWA measured at a right frontal site (F4), more weakly at a left central (C3), and no correlation at a right posterior site (O2). Results for F3, C4 and O1 were not reported in that paper. Slow waves are most prominent frontally, and this may be why low resolution EEG revealed significant associations in that region, and not at more posterior sites where slow wave power is lower. In this study, although the trending association of posterior SWA with CSF biomarkers did not survive correction for multiple comparisons, it was seen consistently across biomarkers and the region stood out clearly against background activity. It could be viewed as an exaggeration of the normal topography of slow waves, which are normally frontally dominant, and lower in posterior regions. This relationship warrants further study in a larger sample.

The group studied here was considerably younger (mean age 60 years) than previous studies, demonstrating that the link between slow wave activity and AD biomarkers is present very early in the preclinical phase, when participants are still cognitively healthy, and biomarkers are still within normal limits. Although this observational study does not allow causal inference, there is evidence that deficits in sleeping brain activity could be both a symptom and a driver of AD pathology (Roh *et al.* 2012; Mander *et al.* 2015), presenting opportunities for detection and intervention. Regional signatures of slow wave deficits may be a very early biomarker of AD, which could facilitate early intervention. Several technologies are under development to enhance slow waves, motivated by the fundamental role slow waves play in cognition and memory (Walker and Stickgold 2006). In preclinical AD, slow wave enhancement might not only bolster cognition, but also actively prevent the accumulation of pathology.

This study is limited by a small sample size (n=21) that was predominantly female (n=20). Given that slow wave dynamics are influenced by sex (Baker *et al.* 2012; Plante *et al.* 2012), it will be important to replicate these findings in a larger sample, and one that includes more men.

CHAPTER 6

General Discussion

GENERAL DISCUSSION

The studies comprising this dissertation aimed to investigate the role of sleep in development of Alzheimer's disease pathology in the preclinical stage. The work in this thesis establishes a link between sleep quality (measured both objectively and subjectively) and AD pathology in middle aged adults who are cognitively healthy. It also describes the changes in topography of sleeping brain activity across healthy aging.

We found that self-report of inadequate, unrefreshing sleep and daytime sleepiness is associated with greater AD pathology, including cortical amyloid burden (measured by PET imaging and in CSF), axonal degeneration and neuro-inflammation. We observed distinct changes in sleeping brain activity in relation to normal aging compared to Alzheimer's disease pathology. Over the course of normal aging, changes in topography varied by EEG frequency band. Slow wave power declined across the entire scalp, whereas theta and sigma power declined in frontal regions, and no age-related changes in alpha power were seen. In contrast to the scalp-wide decline of slow wave power in normal aging, Alzheimer's disease pathology was associated with changes in circumscribed frontal and parietal regions.

There are several potential mechanisms through which sleep may promote AD. Clearance of exogenous amyloid from the brain via the glymphatic system was observed to increase during sleep in mice (Xie *et al.* 2013), and in mice and young men, sleep deprivation promoted amyloid accumulation (Kang *et al.* 2009; Ooms *et al.* 2014). Therefore our findings of greater amyloid burden in poor sleepers may reflect impaired amyloid clearance. Sleep is also a critical time for cell maintenance and repair (Cirelli 2009) and sleep restriction promotes pro-inflammatory cytokines (Faraut *et al.* 2012), which could underly our observation that poor sleepers had greater axonal degeneration and neuroinflammation. Finally, slow wave sleep is known to support cognitive function, therefore loss of SWA could contribute to cognitive decline. Indeed,

one study of older adults reported that diminished frontal slow waves mediated the association of greater cortical amyloid burden with memory deficits (Mander *et al.* 2015).

On the other hand, there are several pathways through which AD pathology may disrupt sleep. In mice and drosophila, sleep is increasingly altered as amyloid and tau pathology develop (Roh *et al.* 2012; Tabuchi *et al.* 2015; Holth *et al.* 2017) and, in the case of amyloid, the changes can be reversed by Aβ immunization (Roh *et al.* 2012), providing evidence that AD pathology can induce sleep disturbances. AD pathology may drive sleep changes by inducing synaptic and network dysfunction (Busche *et al.* 2008; Palop and Mucke 2010; Roberson *et al.* 2011). Such dysfunction could underly the changes we observed in slow wave topography, in relation to CSF biomarkers of amyloid and tau. Additionally, early amyloid and tau pathology are observed in sleep-regulating brain centers including the ventrolateral preoptic nucleus and many components of the ascending arousal system (Braak *et al.* 2011). This could account for our observations of poor sleep in individuals with greater CSF evidence of amyloid and tau.

Importantly, this dissertation research showed that disturbed sleep is related to Alzheimer's pathology in cognitively healthy adults in late midlife. Trials of therapeutics after dementia onset have been unsuccessful in modifying the disease, and the preclinical phase may be a more optimal window for intervention, when neurodegeneration is not yet advanced. Successful preclinical treatments will require strategies for identifying patients who are candidates for treatment, and modifiable risk factors to target with interventions. Given that evidence supports a bidirectional relationship between sleep and AD pathology, sleep may harbor both biomarkers and treatment targets at this early stage.

To the extent that sleep deficits promote AD pathology, sleep may present an excellent target for intervention. 25-30% of US adults have a sleep disorder (National Center on Sleep Disorders Research 2011) and many effective FDA-approved methods already exist to improve sleep.

Optimizing sleep is also beneficial to older adults without dementia, including improved health, cognition and quality of life, and reduced healthcare costs (Gooneratne and Vitiello 2014; McMillan *et al.* 2014, 2015; Crawford-Achour *et al.* 2015). Therefore optimizing sleep is a feasible and potentially fruitful strategy to both address AD and support healthy aging. Sleep interventions could include management of sleep disorders that are undertreated in older adults, such as obstructive sleep apnea, insomnia and circadian dysrhythmias. Other interventions that are currently under development may be useful in preclinical AD, such as technologies for enhancing slow waves to support learning and memory. Some of these techniques have already been shown to benefit learning and memory in older adults with dementia (Ancoli-Israel *et al.* 2003; Cooke *et al.* 2009b; Hu *et al.* 2016), but their ability to impact AD pathology has not yet been explored.

To the extent that AD pathology impairs sleep, sleep characteristics could be useful as AD biomarkers. These could be applied to early diagnosis and prognosis, to enriching clinical trials with patients likely to develop AD, and as outcome measures in intervention trials. The loss of frontal slow wave activity was distinct from the pattern of normal aging, and has been shown by others to correspond to memory function. Slow wave topography may therefore be useful as an early biomarker of AD.

To further develop sleep interventions and biomarkers it will be essential to further clarify the role of sleep in AD pathogenesis. The data collected during this thesis forms a rich foundation for further investigation of sleep in relation to AD. One avenue of particular interest is the role of sleep disordered breathing in promoting early AD pathology. OSA is a disorder in which the airway closes repeatedly during sleep, producing sleep fragmentation and hypoxemia. OSA is common in middle-aged and older adults (Peppard *et al.* 2013) and is a risk factor for AD (Yaffe *et al.* 2011; Yaffe, Nettiksimmons and Byers 2014). Both sleep fragmentation and hypoxia have

been shown to increase amyloid deposition in rodents (Kang *et al.* 2009; Shiota *et al.* 2013a) and cognitive impairment in humans (Wallace and Bucks 2013).

Sleep health may represent an untapped opportunity for detection and treatment of AD. This dissertation research work is a first step towards identifying sleep factors associated with preclinical AD, and is an essential starting point for characterizing sleep-based biomarkers for preclinical AD detection and/or sleep-based treatment targets, enrichment of clinical trial samples, and for generating testable hypotheses about disease mechanisms. It is estimated that a treatment to delay AD by a mere 5 years would result in 5.7 million fewer AD cases and save $367 billion in healthcare costs in the US (Alzheimer's Association 2015). Many effective pharmaceuticals, devices and behavioral interventions are already available in the clinic for improving sleep quality. Follow-up studies are needed to further identify the aspects of sleep that are most amenable to modification, sleep interventions that are most effective in impacting AD pathology and the patients that will benefit most from treatment, to ultimately delay AD or diminish AD symptoms.

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