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REPORT

Slow wave sleep disruption increases cerebrospinal fluid amyloid-β levels

Yo-El S. Ju,[1,2,*] Sharon J. Ooms,[3,4,5,*] Courtney Sutphen,[1,2] Shannon L. Macauley,[1,2] Margaret A. Zangrilli,[1] Gina Jerome,[1,2] Anne M. Fagan,[1,2,6] Emmanuel Mignot,[7] John M. Zempel,[1] Jurgen A.H.R. Claassen[3,4,5] and David M. Holtzman[1,2,6]

*These authors contributed equally to this work
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Sleep deprivation increases amyloid-β, suggesting that chronically disrupted sleep may promote amyloid plaques and other downstream Alzheimer's disease pathologies including tauopathy or inflammation. To date, studies have not examined which aspect of sleep modulates amyloid-β or other Alzheimer's disease biomarkers. Seventeen healthy adults (age 35–65 years) without sleep disorders underwent 5–14 days of actigraphy, followed by slow wave activity disruption during polysomnogram, and cerebrospinal fluid collection the following morning for measurement of amyloid-β, tau, total protein, YKL-40, and hypocretin. Data were compared to an identical protocol, with a sham condition during polysomnogram. Specific disruption of slow wave activity correlated with an increase in amyloid-β_{40} ($r = 0.610$, $P = 0.009$). This effect was specific for slow wave activity, and not for sleep duration or efficiency. This effect was also specific to amyloid-β, and not total protein, tau, YKL-40, or hypocretin. Additionally, worse home sleep quality, as measured by sleep efficiency by actigraphy in the six nights preceding lumbar punctures, was associated with higher tau ($r = 0.543$, $P = 0.045$). Slow wave activity disruption increases amyloid-β levels acutely, and poorer sleep quality over several days increases tau. These effects are specific to neuronally-derived proteins, which suggests they are likely driven by changes in neuronal activity during disrupted sleep.

1 Department of Neurology, Washington University, Saint Louis, Missouri, USA
2 Hope Center for Neurological Disorders, Washington University, Saint Louis, Missouri, USA
3 Department of Geriatric Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands
4 Radboudumc Alzheimer Centre, Nijmegen, The Netherlands
5 Donders Institute for Brain, Cognition and Behaviour, Nijmegen, The Netherlands
6 Knight Alzheimer's Disease Research Center, Washington University, Saint Louis, Missouri, USA
7 Stanford Center for Sleep Sciences and Medicine, Stanford University, Palo Alto, California, USA

Correspondence to: Yo-El S. Ju MD
660 South Euclid Avenue #8111
Washington University School of Medicine
Saint Louis, MO 63110
USA
E-mail: juy@wustl.edu

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Abbreviations: N1/2/3 = non-rapid eye movement sleep stage 1/2/3; SWA = slow wave activity

Introduction

Alzheimer's disease pathology is associated with sleep disruption, even in the preclinical stages of disease (Ju *et al.*, 2013; Spira *et al.*, 2013). Amyloid plaque formation, an early necessary step in Alzheimer's disease pathogenesis, is associated with sleep disruption in a bi-directional manner (Ju *et al.*, 2014). Soluble amyloid-β varies diurnally in both mouse and human (Kang *et al.*, 2009; Huang *et al.*, 2012), where sleep is associated with decreased amyloid-β, and wakefulness with increased amyloid-β. Sleep deprivation acutely increases soluble amyloid-β and chronically increases amyloid plaques in mouse (Kang *et al.*, 2009; Roh *et al.*, 2014), and one night of total sleep deprivation increases soluble amyloid-β in humans (Ooms *et al.*, 2014). However, it is unknown which aspect of sleep is responsible for modulation of amyloid-β. Slow wave activity (SWA), which occurs during deep non-REM sleep, is a strong candidate for amyloid-β modulation. The EEG slow waves that characterize SWA represent decreased synaptic activity (Nir *et al.*, 2011), and soluble amyloid-β is released into the interstitial space during neuronal synaptic activity (Cirrito *et al.*, 2005). Furthermore, clearance of solutes from the interstitial space, including exogenous amyloid-β, accelerates during SWA-rich sleep (Xie *et al.*, 2013). These data lead to the hypothesis that disrupted SWA would cause relatively increased neuronal activity, increased amyloid-β release and decreased clearance, resulting in increased amyloid-β levels measurable in the CSF. Indeed, in cross-sectional studies, SWA was negatively correlated with CSF amyloid-β in middle-aged (Ju *et al.*, 2016) and older (Varga *et al.*, 2016) individuals. Furthermore, chronic sleep disruption increases tau levels and tau phosphorylation in mice (Rothman *et al.*, 2013; Qiu *et al.*, 2016). Tau is also released during neuronal activity, although clearance from the brain is slower than for amyloid-β (Yamada *et al.*, 2014).

In this study, we used a novel SWA disruption protocol to test whether specific disruption of SWA leads to increased amyloid-β and tau levels in CSF.

Materials and methods

Participants

We enrolled 22 participants aged 35–65 years from a community-based research registry at Washington University. Participants had no comorbidities except controlled hypertension, took no neuro-active medications, had normal neurological exams, reported $\leqslant 14$ alcoholic beverages weekly, had body mass index 18–40 kg/m^2, and were cognitively normal based on history, neurological examination, and Mini-Mental State Examination (MMSE) $\geqslant 27/30$ (Folstein *et al.*, 2004). Participants had regular sleep schedules with bedtime 8 pm–12 am and wake time 4 am–8 pm. Participants did not have obstructive sleep apnoea or periodic limb movement disorder, defined as apnoea-hypopnoea index $\geqslant 5$ or periodic limb movement index $\geqslant 15$, during a screening polysomnogram. All participants provided informed, written consent. All procedures were approved by the Washington University Human Research Protection Office.

Experimental design

All participants underwent two sets of procedures, $\geqslant 28$ days apart. Each procedure set consisted of a daytime visit with questionnaires, actigraphy for 5–14 days, overnight polysomnogram with experimental condition, and lumbar puncture the following morning. An automated SWA disruption protocol was applied for one polysomnogram, and a sham condition was used for the other, in random order. SWA disruption was performed with parameters and methods determined to be effective in this age group (Ooms *et al.*, 2017). Briefly, every 10 s, EEG data were extracted from the live recording. If the data were determined be non-artefactual, and 0.5–4 Hz spectral power was $> 100 \mu V^2 \times s$ indicating SWA, a tone was delivered through earphones to the participant. The amplitude of the tones would progressively increase, until delta power decreased, indicating an arousal out of SWA. During sham condition, participants wore earphones but no tones were delivered. The order of the two conditions was random, and participants were blinded to the condition. All analyses were conducted blinded to condition.

Sleep and EEG spectral analysis

Polysomnograms and sleep staging by a registered polysomnographic technologist were performed according to standard criteria (Iber, 2007). Lights out was at 10 pm, lights on at 6 am. SWA was quantified as spectral power in the delta (0.5–4 Hz) band, or 'delta power', averaged over all non-REM epochs, from bilateral frontal and central electrodes. EEG collection and analysis parameters are detailed in the Supplementary material.

Actigraphy data were collected using Actiwatch2™ (Philips-Respironics) and scored as previously described (Ju *et al.*, 2013; see Supplementary material). Participants were instructed to keep regular sleep schedules prior to polysomnograms. If actigraphy did not demonstrate $\geqslant 6$ h in bed and bedtime 8 pm–12 am on the night prior to either polysomnogram, the participant was excluded. This criterion excluded four participants.

To assess home sleep, actigraphy data from six nights (polysomnogram night and five preceding nights) were scored using polysomnographically-validated criteria (Kushida *et al.*, 2001) to derive total sleep time and sleep efficiency (total sleep time divided by time in bed). Time in bed was calculated as minutes between bedtime and wake time, mid-sleep as the halfway-point between bedtime and wake time. Three participants wore a defective actigraph that prevented determination of total sleep time and sleep efficiency.

CSF analytes and *APOE* genotype

CSF was obtained by lumbar puncture at 9:30–10 am following polysomnography. This time corresponds to the daily nadir of CSF amyloid-β$_{40}$ (Huang *et al.*, 2012), and follows the sleep mid-point by ~6 h, the transit time of amyloid-β from CNS to

CSF (Bateman *et al.*, 2006). CSF was immediately placed on ice, aliquoted, and frozen at $-80°C$. Amyloid-β_{40} was chosen as the primary measure of amyloid-β since it is the most abundant amyloid-β species and less subject to altered levels caused by amyloid plaques. Amyloid-β_{40}, amyloid-β_{42}, and tau, were assessed by INNOTEST® ELISA (Fujirebio). Total protein was assessed by Bradford assay (Thermo Pierce). YKL-40, a glial marker of neuroinflammation, was assessed by MicroVue ELISA (Quidel) (Sutphen *et al.*, 2015). Hypocretin-1 was assessed by radioimmunoassay as previously described (Mignot *et al.*, 2002). All CSF measurements were performed in duplicate or triplicate with several internal control samples.

As existing amyloid plaques abolish the diurnal variation of amyloid-β related to sleep, (Huang *et al.*, 2012; Roh *et al.*, 2012), individuals with abnormally low amyloid-β_{42} levels (<608 pg/ml) indicating amyloid plaques were excluded. Cutoff level was determined by the Knight Alzheimer's Disease Research Center Biomarker Core by comparison of amyloid-β_{42} values and Pittsburgh compound B PET data from large cohorts (Fagan *et al.*, 2006; Schindler *et al.*, 2016). Two participants were excluded based on this criterion; one had already been excluded for inadequate actigraphically-measured sleep.

Fasted blood (20 ml) was drawn following lumbar puncture, and placed on ice until centrifugation and storage at $-80°C$. The Hope Center DNA/RNA Purification Core performed DNA extraction and *APOE* genotyping. Genotype was dichotomized as *APOE* $\varepsilon 4$ carrier or non-carrier.

Questionnaires

The Epworth Sleepiness Scale (Johns, 1991) and Pittsburgh Sleep Quality Index (Buysse *et al.*, 1989) were completed during the daytime visits 5–14 days prior to each polysomnogram to assess sleepiness and sleep quality, respectively. Immediately following each polysomnogram, participants completed the Stanford Sleepiness Scale (Hoddes *et al.*, 1973), a measure of immediate subjective sleepiness. They also completed a written questionnaire which asked: 'Do you recall being woken during the night because of the noises through the earphones?'; 'If yes, how many times do you think you woke up because of the noises?'; estimated sleep time in hours; and Sleep quality on a scale of 1–10 (10 best).

Statistical analysis

All continuous variables were assessed for normal distribution by inspection of histograms and the Kolmogorov-Smirnov test. Levene's test was used to assess equality of variances. Differences between SWA disruption and sham conditions for continuous variables were calculated using paired *t*-tests for normally-distributed variables, and related-samples Wilcoxon signed rank test for other variables. Correlations were assessed with Spearman's correlation coefficient. To compare participants grouped by order of polysomnogram condition, *APOE* genotype, or response to SWA disruption, unpaired *t*-tests were used to compare continuous variables and Fisher exact tests were used to compare dichotomous variables. Tests were two-tailed, and α was 0.05. Statistical analyses were performed with SPSS 24 (IBM). Figures were generated in GraphPad Prism (GraphPad Software Inc).

As interindividual amyloid-β variation ($\sim30\%$) is much greater than intraindividual amyloid-β variation ($\sim10\%$), and may be subject to *APOE* genotype (Osorio *et al.*, 2014), a repeated-measures design was selected. From preliminary cross-sectional data, the effect size for variance in amyloid-β_{40} explained by SWA was f = 0.25. Assuming a moderate (0.7) correlation between repeated measures, α 0.05, and power 0.8, a total sample size of 20 was calculated. We stopped enrolment when 20 participants had completed the study, and two others already enrolled were permitted to complete the study, for a total of 22 participants.

Results

Twenty-two participants completed the study; five participants were excluded for predefined exclusion criteria, for a sample size of 17. The study population was middle-aged (54.1 ± 6.7 years) with a slight majority Caucasian race and female sex (65% each), and five (29%) were *APOE* $\varepsilon 4$ carriers. Questionnaire-based sleep measures were in the normal range and actigraphy showed normal home sleep patterns (Table 1).

The SWA disruption protocol decreased SWA as measured by delta power by 23 (95% confidence interval 14–32) $\mu V^2 \times s$. As expected, there was decreased N3 sleep with a compensatory increase in N1 sleep during the SWA disruption night, and a decrease in REM sleep; otherwise there was no difference in other polysomnographic sleep variables (Table 1). The participants who underwent SWA disruption first were similar to the participants who underwent sham condition first (Supplementary Table 1). Participants estimated sleep was of shorter duration and worse quality with SWA disruption, although subjective sleepiness was no different in the morning (Table 1).

SWA disruption was strongly and significantly correlated with amyloid-β_{40}, such that more SWA disruption was associated with greater increases in amyloid-β_{40} (Fig. 1A). This effect was specific for SWA disruption, and not for total sleep time, non-REM time, REM time, or sleep efficiency (Fig. 1B–E). While in the entire group there was no significant difference in amyloid-β_{40} levels between conditions, the subset with sufficient SWA disruption (greater than median, or $\geqslant 20\,\mu V^2 \times s$ decrease) had a significant increase in amyloid-β_{40} (Fig. 1F). These 'responders' to the SWA disruption protocol essentially drove the correlation between SWA disruption and amyloid-β_{40} change.

We then assessed whether the effect of SWA on amyloid-β_{40} was specific to amyloid-β. There was no correlation between SWA disruption and change in total protein (Fig. 2A). YKL-40 (an astrocyte-derived inflammatory protein) and tau (a neuronally-derived protein), both biomarkers increased in Alzheimer's disease, were not correlated with SWA disruption (Fig. 2B and C). Hypocretin, a wake-promoting peptide that may modulate amyloid-β kinetics and is increased after REM sleep deprivation (Pedrazzoli *et al.*, 2004; Roh *et al.*, 2014), was not correlated with SWA disruption (Fig. 2D). Supporting the relationship between SWA and amyloid-β, in addition to the strong correlation between SWA and amyloid-β_{40}, SWA disruption was also

Table 1 Demographic and sleep characteristics

	Sham condition Mean ± SD	SWA disruption Mean ± SD	P-value
Demographics			
Age (years)	54.1 ± 6.7	n/a	
Race White n (%)	11 (65)	n/a	
Sex female n (%)	11 (65)	n/a	
Body mass index (kg/m^2)	28.2 ± 4.3	n/a	
Education (years)	15.8 ± 1.6	n/a	
Alcohol (drinks/week)	1.6 ± 2.1	n/a	
MMSE	29.6 ± 0.5	n/a	
APOE ε4 carrier n (%)	5 (29)	n/a	
Polysomnogram			
Total sleep time (min)	419 ± 47	404 ± 51	0.160
non-REM sleep (min)	311 ± 39	300 ± 49	0.244
N1 (min)	22 ± 10	34 ± 18	**0.007**
N2 (min)	265 ± 44	254 ± 43	0.33
N3 (min)	7 ± 42[a]	0 ± 2[a]	**<0.001**[a]
REM (min)	99 ± 52[a]	68 ± 38[a]	**0.001**[a]
Sleep efficiency (%)	85.4 ± 8.5	82.4 ± 9.6	0.102
Number tones delivered	0	1177 ± 427	n/a
EEG spectral power			
0.5–4 Hz (Delta) (μV^2 × s)	118 ± 51	96 ± 43	**<0.001**
4–8 Hz (μV^2 × s)	12.8 ± 5.4	12.0 ± 5.4	0.215
8–12 Hz (μV^2 × s)	6.2 ± 3.0	6.4 ± 3.7	0.535
12–18 Hz (μV^2 × s)	2.7 ± 1.2	3.0 ± 1.3	**0.005**
18–32 Hz (μV^2 × s)	1.7 ± 0.8	2.1 ± 0.9	**0.008**
Actigraphy (n = 14)			
Total sleep time (min), mean six nights	377 ± 56	370 ± 43	0.445
Total sleep time (min), polysomnogram night	422 ± 42	404 ± 41	0.117
Sleep efficiency (%), mean six nights	82.6 ± 7.5	82.0 ± 6.7	0.655
Time in bed (min)[b]	456 ± 46	454 ± 37	0.836
Mid-sleep (hh:min)[b]	03:48 am ± 00:23	03:47 am ± 00:19	0.836
Questionnaires			
Pittsburgh Sleep Quality Index[c]	4.2 ± 1.6	3.4 ± 1.8	0.059
Epworth Sleepiness Scale[c]	5.6 ± 3.6	6.4 ± 3.4	0.133
Awakened by noise? (yes)[d]	1/16	12/15	**<0.001**
Number tones heard?[d]	3 (n = 1)	11.1 ± 8.9	n/a
Estimated sleep hours	7.3 ± 1.1	5.2 ± 2	**<0.001**
Estimated sleep quality	7.4 ± 1.7	5.5 ± 2.7	**0.02**
Stanford Sleepiness Scale	2.0 ± 0.9	2.8 ± 1.8	0.16

[a]Median ± interquartile range; P-value is for related-samples Wilcoxon signed rank test.
[b]n = 17.
[c]Completed during daytime 5–14 days prior to polysomnogram nights.
[d]Not all participants answered this question.

strongly correlated with amyloid-β$_{42}$ (Supplementary Fig. 1). The CSF amyloid-β$_{42}$:amyloid-β$_{40}$ ratio decreased with greater SWA disruption, but this was not significant (Supplementary Fig. 1).

Extracellular levels of tau in the brain, like amyloid-β, are regulated by neuronal activity, but the half-life of tau is longer, 11 days in the mouse brain (Yamada et al., 2014). As SWA disruption did not lead to changes in tau the following morning, we compared actigraphically-measured home sleep over six preceding nights to CSF tau levels. There was a significant negative correlation between home sleep quality and tau, such that worse sleep efficiency was associated with higher tau (Fig. 3A). Sleep quantity

was not correlated with tau (Fig. 3B). A strong trend for a correlation between home sleep quality and amyloid-β$_{40}$ was apparent (Fig. 3C); however, this effect was likely diminished by SWA disruption immediately prior to the lumbar punctures. There was no association between amyloid-β$_{40}$ and sleep quantity, or between total protein and either sleep quantity or quality (Fig. 3D–F).

Discussion

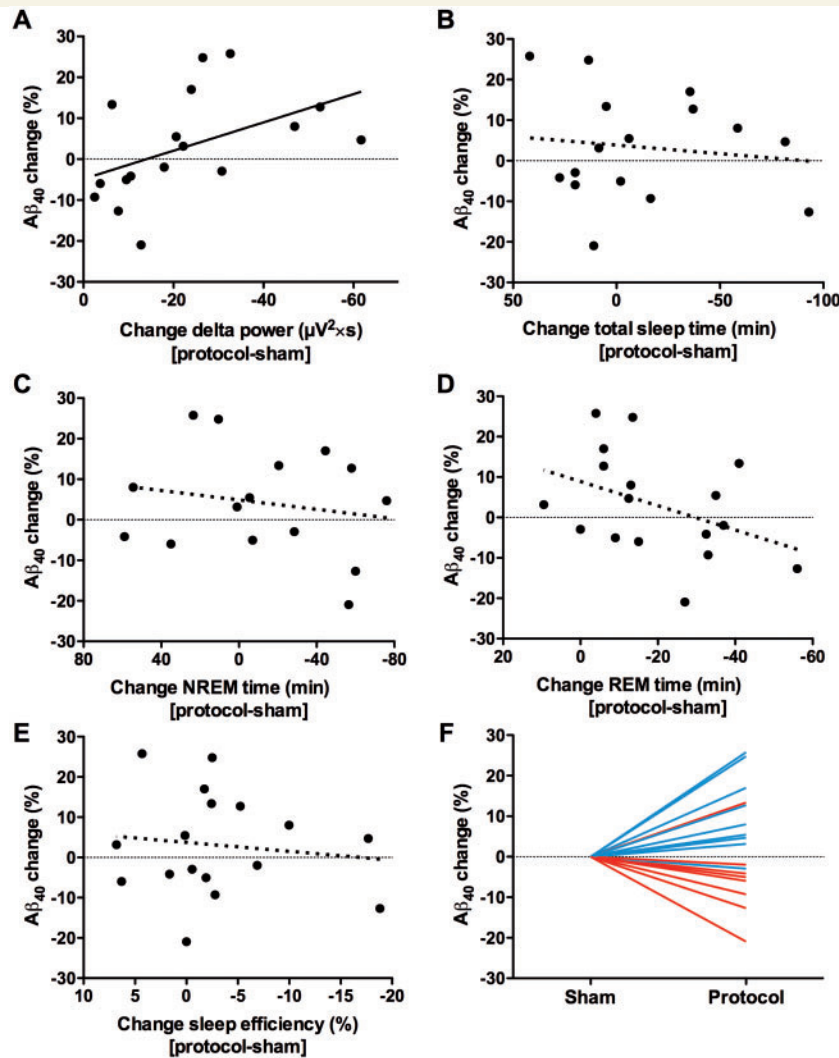Our hypothesis was that one night of selective SWA disruption would lead to increased CSF amyloid-β the

Figure 1 Decreased slow wave activity is associated with increased CSF amyloid-β. (**A**) Suppression of slow wave activity, as measured by the change in delta spectral power, was strongly correlated with increased amyloid-β_{40} ($r = 0.610$, $P = 0.009$). There was no correlation between change in amyloid-β_{40} levels and (**B**) total sleep time ($r = -0.075$, $P = 0.782$), (**C**) time in non-REM sleep ($r = -0.156$, $P = 0.564$), (**D**) time in REM sleep ($r = -0.351$ $P = 0.168$), or (**E**) sleep efficiency ($r = -0.007$, $P = 0.978$). (**F**) When participants are divided at the median ($20\,\mu V^2 \times s$) amount of slow wave activity disruption, the 'responders' to SWA disruption (blue lines) had a significant increase in amyloid-β_{40} ($n = 9$, 11562 ± 2603 versus 10562 ± 2868 pg/ml, 95% confidence interval difference 315 to 1686 pg/ml, $P = 0.010$) while 'non-responders' (red lines) did not. X-axes in **A–E** are more negative to the right, i.e. values to the right indicate more disruption of slow wave activity or less sleep.

following morning. We found a strong association between SWA and amyloid-β, where greater SWA disruption was associated with increased amyloid-β. This effect was specific for SWA and not sleep quantity or quality, and was also specific for amyloid-β but not other CSF proteins. Additionally, we found that worse home sleep quality was associated with increased CSF tau, consistent with sleep disruption affecting CNS tau, but with such changes requiring a longer time to detect likely due to the longer half-life of tau.

Both of these findings support the hypothetical cascade in which decreased SWA leads to increased soluble amyloid-β and tau levels, which eventually increase risk of amyloid

plaques (Bero et al., 2011) and tau tangle pathology, and subsequent development of symptomatic Alzheimer's disease. Furthermore, the specificity of our findings for neuronally-derived proteins (amyloid-β and tau) and not for the glial-derived YKL-40 nor for total CSF protein levels suggests that SWA affects amyloid-β and tau by influencing synaptic activity with decreased release of these proteins into the interstitial space, rather than by affecting general protein clearance mechanisms. That total protein levels in CSF were unaffected suggests there were no global effects of SWA disruption on bulk flow mechanisms by which albumin and other abundant proteins enter and exit the CNS. While the CSF amyloid-β_{42}:amyloid-β_{40} ratio
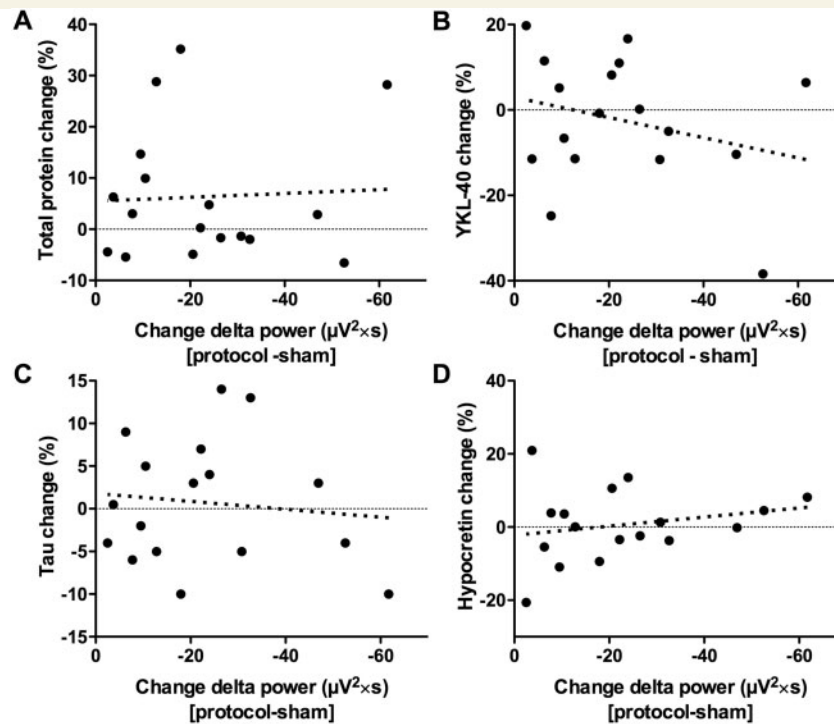
Figure 2 Slow wave activity disruption is not correlated with change in other CSF proteins. SWA disruption, as measured by the change in delta spectral power, was not correlated with change in (**A**) total protein ($r = 0.098$, $P = 0.708$), (**B**) YKL-40 ($r = -0.199$, $P = 0.445$), (**C**) tau ($r = 0.000$, $P = 1.000$), or (**D**) hypocretin ($r = -0.250$, $P = 0.333$). X-axes are more negative to the right, i.e. values to the right indicate more disruption of slow wave activity.

tended to decrease with greater SWA disruption, this finding was not significant and the study was not powered or designed to assess relative clearance of amyloid-β isoforms. It is possible that following amyloid deposition, the relative clearance of amyloid-β_{42} from the brain's interstitial fluid space to the CSF would be retarded by sequestration of monomeric amyloid-β_{42} into plaques (Potter *et al.*, 2013), which could influence the effect of SWA disruption on CSF amyloid-β_{42}.

A weakness of the study is the relatively small sample size. However, the effect size was stronger than predicted by cross-sectional preliminary data, with a high *r* of 0.610 for the association between SWA disruption and amyloid-β_{40} change. In addition, intraindividual analyses strongly increased statistical power. Nevertheless, our study was not *a priori* powered to assess correlations between SWA disruption and other CSF proteins, and larger studies are necessary to definitively exclude a relationship between SWA and other CSF proteins. Additionally, determining the relative clearance rates of amyloid-β isoforms, and any effect of amyloid plaques, will require larger studies including amyloid-positive individuals. Similarly, due to the small ($n = 5$) number of *APOE* ε4 carriers, this study was underpowered to assess the effect of *APOE* genotype on susceptibility to SWA disruption (Supplementary Table 2). Another weakness is that SWA disruption did

not increase amyloid-β in some participants. There were no differences between 'responders' and 'non-responders' (Supplementary Table 3) to the SWA disruption protocol except that 'non-responders' had lower baseline SWA during the sham night (delta power 86 versus $147 \mu V^2 \times s$, $P = 0.009$). Since the same delta power $> 100 \mu V^2 \times s$ cut-off was used in all participants for delivery of tones during SWA disruption, low baseline SWA made it difficult to reduce SWA further, and therefore blunted any effect on amyloid-β. Another minor weakness is that amyloid imaging was not used to exclude individuals with amyloid deposition; however, low CSF amyloid-β_{42} levels correlate well with amyloid deposition detected by PET (Fagan *et al.*, 2006, 2009). Lastly, this experiment tested only the effect of SWA disruption; we cannot infer any information about the effect of increasing SWA on synaptic activity or protein clearance from this experiment.

Our study supports the hypothesis that SWA and sleep quality modulate amyloid-β and tau levels, respectively. Prospective studies will be required to test whether improving sleep quality and increasing SWA by treating underlying sleep disorders, medications, behavioural interventions, or acoustic enhancement of SWA can reduce amyloid-β levels, long-term risk of amyloid deposition, and progression to Alzheimer's disease.
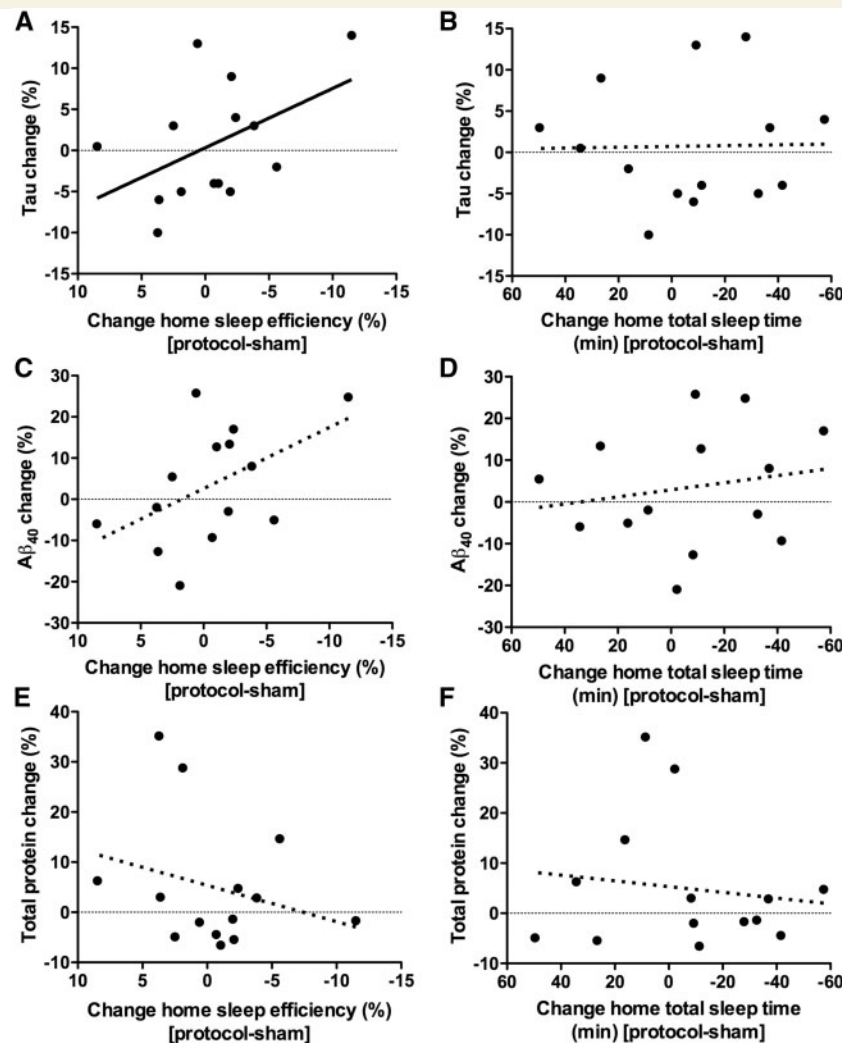
Figure 3 Worse home sleep quality over six nights is associated with increased CSF tau. Home sleep was quantified by actigraphically-measured sleep variables for the six nights prior to lumbar punctures. Valid actigraphy data were available for $n = 14$ participants. For consistency with other figures, sleep variables are shown as the six nights that included SWA disruption protocol night minus the six nights that included the sham condition night; however, the differences in total sleep time or sleep efficiency reflect variations in home sleep and are not related to the protocol or sham condition. For consistency with other figures, x-axes are more negative to the right, i.e. values to the right indicate less or worse sleep. (**A**) Worse sleep quality, as measured as the sleep efficiency, was associated with greater tau levels ($r = 0.543$, $P = 0.045$). (**C**) There was a strong but non-significant trend for an association between worse home sleep quality and amyloid-β_{40} levels ($r = 0.481$, $P = 0.081$), but (**E**) there was no correlation between sleep quality and total protein ($r = -0.218$, $P = 0.455$). Change in home sleep quantity, as measured by actigraphically-determined total sleep time, had no correlation with (**B**) tau ($r = 0.103$, $P = 0.725$), (**D**) amyloid-β_{40} ($r = 0.218$, $P = 0.455$), or (**F**) total protein ($r = -0.103$, $P = 0.725$).

Supplementary material

Supplementary material is available at *Brain* online.

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