Neuroinflammation and Tau Interact with Amyloid in Predicting Sleep Problems in Aging Independently of Atrophy

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Abstract

Sleep problems relate to brain changes in aging and disease, but the mechanisms are unknown. Studies suggest a relationship between β-amyloid (Aβ) accumulation and sleep, which is likely augmented by interactions with multiple variables. Here, we tested how different cerebrospinal fluid (CSF) biomarkers for brain pathophysiology, brain atrophy, memory function, and depressive symptoms predicted self-reported sleep patterns in 91 cognitively healthy older adults over a 3-year period. The results showed that CSF levels of total- and phosphorylated (P) tau, and YKL-40—a marker of neuroinflammation/astroglial activation—predicted poor sleep in Aβ positive older adults. Interestingly, although brain atrophy was strongly predictive of poor sleep, the relationships between CSF biomarkers and sleep were completely independent of atrophy. A joint analysis showed that unique variance in sleep was explained by P-tau and the P-tau × Aβ interaction, memory function, depressive symptoms, and brain atrophy. The results demonstrate that sleep relates to a range of different pathophysiological processes, underscoring the importance of understanding its impact on neurocognitive changes in aging and people with increased risk of Alzheimer’s disease.
Introduction

Sleep problems may be both causative and indicative of brain changes in normal aging (Scullin and Blwise 2015) and age-related degenerative conditions (Prinz et al. 1982; Hatfield et al. 2004; Videnovic et al. 2014). Hence, it is of critical importance to understand the nature of the relationship between sleep problems on one hand, and brain biomarkers and cognitive and emotional function on the other. Especially interesting is the putative relationship between sleep and β-amyloid (Aβ), a key Alzheimer’s disease (AD) biomarker. Sleep disturbances may drive pathogenesis early in the course of neurodegeneration (Musiek and Holtzman 2016), but evidence also indicates that Aβ accumulation can cause sleep problems (Brown, Rainey-Smith, Bucks, et al. 2016), which again may reduce the brains ability to clear Aβ in a positive feedback loop. Several studies have reported that sleep problems are associated with accumulation of Aβ even in healthy older adults (Ju et al. 2013; Spira et al. 2013; Mander et al. 2015; Sprecher et al. 2015; Branger et al. 2016; Brown, Rainey-Smith, Bucks, et al. 2016; Brown, Rainey-Smith, Villemagne, et al. 2016), but the relationships are usually relatively weak, with different sleep parameters affected across studies. Combined with the multi-factorial nature of age-related degenerative diseases (Herrup 2010; Jagust 2013), this implies that it is necessary to explore mechanisms working in synergy with Aβ to cause sleep problems. We approached this challenge by testing whether cerebrospinal fluid (CSF) biomarkers of relevance for sleep problems interacted with Aβ in predicting sleep quality over a 2-year period in cognitively healthy older adults.

First, a tau, marker for axonal degeneration (Blenow et al. 1995), may be of special relevance to sleep (Mander et al. 2016). Neurofibrillary tangles—consisting of an abnormally hyperphosphorylated form of tau—originate in the medial temporal lobe (MTL) (Braak and Braak 1985), and hippocampus is critical for nonrapid eye movement sleep spindles and slow waves supporting sleep-dependent memory processing (Diekelmann and Born 2010; Staresina et al. 2015). Accordingly, some studies found CSF levels of tau to be associated with sleep problems (Liguori et al. 2014, 2016; Osorio et al. 2016). Thus, understanding possible synergistic relationships between Aβ and tau pathology in accounting for sleep problems is a major task (Holth et al. 2017): Is the impact of Aβ and tau on sleep interrelated or independent, and do these interactions forecast the progression of cognitive decline (Mander et al. 2016)? Second, the inflammation and astrogial activation marker YKL-40 (chitinase-3-like protein-1) has been extensively researched in relation to sleep conditions such as obstructive sleep apnea (Jafari et al. 2014; Li et al. 2014; Duru et al. 2015; Sun et al. 2015; Jafari and Mohsenin 2016), but it has not been tested whether neuroinflammation also impact age-related sleep problems. This is an interesting hypothesis because CSF levels of YKL-40 (Craig-Schapiro et al. 2010) increase with age (Schuemaker et al. 2012) and in AD (Craig-Schapiro et al. 2010; Antonell et al. 2014; Rosen et al. 2014; Janelidze et al. 2016) (but see Mattsson et al. 2011), not finding different YKL-40 levels between AD patients and controls). Inflammation is also related to brain atrophy (Alcolea et al. 2015; Gispert et al. 2016) and is assumed to start early in the cascade of neurodegeneration, as damaged neurons, insoluble Aβ deposits and neurofibrillary tangles provide prime stimuli for inflammation (Akiyama et al. 2000; Lee et al. 2008). Thus, it is possible that YKL-40 could interact with Aβ in predicting sleep problems. Third, neurofilament light (NFL), reflecting axonal degeneration (Petzold 2005; Zetterberg et al. 2006), has recently been shown to predict hippocampal atrophy in mild cognitive impairment (Zetterberg et al. 2016) and normal aging (Idland et al. 2016). As argued above, biomarkers related to MTL atrophy are potentially useful tools to help us understand mechanisms for sleep disturbances in aging and neurodegeneration.

In the present study, we tested whether CSF levels of tau, YKL-40, and NFL interacted with Aβ in prediction of sleep quality over a 2-year period. Since even normal aging is associated with brain atrophy (Jell et al. 2009, 2013; Storsve et al. 2014) and sleep problems are related to increased atrophy in older adults (Sexton et al. 2014), we further tested whether CSF biomarkers predicted sleep problems independently of brain atrophy, or indirectly by impacting atrophy rates. Finally, as sleep has consistently been related to levels of depression and memory function (Stickgold and Walker 2013; Mander et al. 2016), these variables were also included in the model.

Materials and Methods

Sample

The study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Committee for Ethics in Medical Research in Norway (REK 2011/2052). All participants provided written informed consent. General recruitment and screening procedures are previously described (Idland et al. 2016). In short, patients were scheduled for elective gynecological (genital prolapse), urological (benign prostate hyperplasia, prostate cancer, or bladder tumor/cancer) or orthopedic (knee or hip replacement) surgery in spinal anesthesia, turning 65 years or older the year of inclusion. Dementia, previous stroke with sequel, Parkinson’s disease, and other neurodegenerative diseases likely to affect cognitive function were initial exclusion criteria. As part of the clinical evaluation, participants were assessed with a multi-domain battery of cognitive tests before surgery, comprising the Mini-Mental Status Examination (MMSE, Folstein et al. 1975), Clock Drawing Test (Shulman 2000), Word List Memory Task (Morris et al. 1989), Trail Making Test A and B (Reitan 1955), Kendrick Object Learning Test (Kendrick et al. 1979), and verbal fluency (FAS test and Animal Naming) (Spreen and Strauss 1991). Also, the Montgomery and Åsberg Depression Rating Scale (MADRS; Montgomery and Åsberg 1979) was administered to assess the level of depressive symptoms. Blood and CSF samples were collected by the anesthesiologist in conjunction with spinal anesthesia, and participants underwent magnetic resonance imaging (MRI) after surgery. One hundred and seventy-two participants were tested at baseline. From this pool of participants, we further selected only cognitively healthy participants based on clinical examinations at Department of Geriatric Medicine. Three participants were excluded due to stroke with sequel, 8 were referred to further cognitive assessment, 6 were not cognitively healthy, and 4 participants...
had CSF NFL levels >4000 pg/mL (i.e., more than ±3 SD from the mean value). After screening, 103 participants had both MRI and CSF data available at baseline, and 91 of these also completed the Pittsburg Sleep Quality Index (PSQI) (mean age 72 years, range 64–89), while longitudinal MRI data were available for 78 of these. Three participants had MMSE (Folstein et al. 1975) score <27 at baseline. None of these dropped more than one point during the 2-year follow-up interval, and the one with a score below 25 at baseline (23) improved to 30 at the follow-up. Thus, these 3 patients were included in the analyses. The mean time between CSF sampling and MRI at baseline was 8 weeks. Participants underwent a second MRI and were tested with the same battery of cognitive tests at 2-year follow-up (mean time between MRIs = 2.2 years, SD = 0.3). Sample characteristics are described in Table 1. An overview of the study design is given in Figure 1.

### MRI Acquisition and Processing

T1-weighted MPRAGE 3D images were acquired with a 1.5 T Siemens Avanto scanner using a 12-channel head coil (time repetition = 2400 ms, time echo = 3.79 ms, field of view = 240 mm, slice thickness = 1.20 mm, pixel size = 1.25 x 1.25 mm). Images were processed with the longitudinal stream in FreeSurfer 5.3 (https://surfer.nmr.mgh.harvard.edu). For each MRI, the FreeSurfer pipeline performs a set of automated procedures for the cortical reconstruction and volumetric segmentation, documented elsewhere (Dale et al. 1999; Fischl et al. 2002). The FreeSurfer longitudinal stream includes methods designed to minimize the bias to any time point which lead to increased statistical power, better separation of groups based on atrophy, and higher reproducibility. These include the generation of a subject-specific intermediate template followed by a projection of each time point to this template (Reuter et al. 2012; Dale et al. 1999; Fischl et al. 2002).

### Table 1 Sample descriptives

<table>
<thead>
<tr>
<th></th>
<th>Participants with CSF and PSQI</th>
<th>Participants with CSF, PSQI and longitudinal MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline</td>
<td>72 (64–89)</td>
<td>72 (64–89)</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>47/44</td>
<td>38/40</td>
</tr>
<tr>
<td>Education (years)</td>
<td>15 (7–23)</td>
<td>14 (7–23)</td>
</tr>
<tr>
<td>MMSE score, baseline</td>
<td>29 (25–30)</td>
<td>29 (25–30)</td>
</tr>
<tr>
<td>MMSE* score, 2-year follow-up</td>
<td>29 (23–30)</td>
<td>29 (23–30)</td>
</tr>
<tr>
<td>MADRS baseline</td>
<td>3.8 (0–15)</td>
<td>3.1 (0–15)</td>
</tr>
<tr>
<td>MADRS*, 2-year follow-up</td>
<td>3.7 (0–16)</td>
<td>2.6 (0–16)</td>
</tr>
<tr>
<td>CERAD 10 words total baseline</td>
<td>20.8 (16–28)</td>
<td>20.8 (16–27)</td>
</tr>
<tr>
<td>CERAD* 10 words total, 2-year follow-up</td>
<td>22.2 (14–29)</td>
<td>22.2 (14–29)</td>
</tr>
<tr>
<td>CERAD 10 words hits, baseline</td>
<td>6.7 (4–10)</td>
<td>6.7 (4–10)</td>
</tr>
<tr>
<td>CERAD* 10 words hits, 2-year follow-up</td>
<td>7.7 (3–10)</td>
<td>7.7 (3–10)</td>
</tr>
<tr>
<td>COWA verbal fluency letters baseline</td>
<td>43.2 (19–69)</td>
<td>43.1 (24–69)</td>
</tr>
<tr>
<td>COWA verbal fluency letters, 2-year follow-up</td>
<td>45.8 (18–77)</td>
<td>45.7 (18–77)</td>
</tr>
<tr>
<td>COWA verbal fluency animals, baseline</td>
<td>20.8 (5–32)</td>
<td>20.6 (5–32)</td>
</tr>
<tr>
<td>COWA verbal fluency animals, 2-year follow-up</td>
<td>23.1 (10–38)</td>
<td>23.2 (10–38)</td>
</tr>
<tr>
<td>Trail Making Test A baseline</td>
<td>50.5 (26–120)</td>
<td>50.9 (26–120)</td>
</tr>
<tr>
<td>Trail Making Test A, 2-year follow-up</td>
<td>47.5 (19–163)</td>
<td>47.8 (19–163)</td>
</tr>
<tr>
<td>Trail Making Test B baseline</td>
<td>122.5 (34–466)</td>
<td>121.1 (34–466)</td>
</tr>
<tr>
<td>Trail Making Test B, 2-year follow-up</td>
<td>113.9 (31–460)</td>
<td>114.9 (31–460)</td>
</tr>
<tr>
<td>CSF Aβ 1–42 pg/mL</td>
<td>732 (202)</td>
<td>736 (200)</td>
</tr>
<tr>
<td>Aβs/Aβ−/Aβ−</td>
<td>23/68</td>
<td>20/58</td>
</tr>
<tr>
<td>CSF T-tau (pg/mL)</td>
<td>363 (130)</td>
<td>367 (135)</td>
</tr>
<tr>
<td>CSF P-tau (pg/mL)</td>
<td>60 (18)</td>
<td>60 (19)</td>
</tr>
<tr>
<td>CSF NFL (pg/mL)</td>
<td>1110 (527)</td>
<td>1119 (547)</td>
</tr>
<tr>
<td>CSF YKL-40 (pg/mL)</td>
<td>218 041 (80 147)</td>
<td>218 853 (83 413)</td>
</tr>
<tr>
<td>APOE* (n with 1–2 ɛ4 allele)</td>
<td>37 (44%)</td>
<td>31 (42%)</td>
</tr>
<tr>
<td>Years between MRIs</td>
<td>2.2 (0.3)</td>
<td>2.2 (0.3)</td>
</tr>
<tr>
<td>BMI</td>
<td>25.9 (3.3)</td>
<td>25.8 (3.1)</td>
</tr>
<tr>
<td>PSQI subscales</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comp 1: Quality</td>
<td>0.95 (0.72)</td>
<td>0.95 (0.66)</td>
</tr>
<tr>
<td>Comp 2: Latency</td>
<td>1.20 (0.89)</td>
<td>1.16 (0.87)</td>
</tr>
<tr>
<td>Comp 3: Duration</td>
<td>0.99 (0.82)</td>
<td>0.97 (0.81)</td>
</tr>
<tr>
<td>Comp 4: Efficiency</td>
<td>1.04 (1.10)</td>
<td>1.04 (1.08)</td>
</tr>
<tr>
<td>Comp 5: Problems</td>
<td>0.99 (0.51)</td>
<td>1.00 (0.48)</td>
</tr>
<tr>
<td>Comp 6: Medication</td>
<td>0.66 (1.11)</td>
<td>0.49 (0.95)</td>
</tr>
<tr>
<td>Comp 7: Tired</td>
<td>0.53 (0.62)</td>
<td>0.51 (0.64)</td>
</tr>
<tr>
<td>Global</td>
<td>6.32 (3.77)</td>
<td>6.08 (3.56)</td>
</tr>
</tbody>
</table>

Numbers in parentheses denotes range or standard deviations. TMT/COWA: available for 87–91.

COWA, Controlled Word Association Test; APOE, the combination ɛ2/ɛ4 was excluded (n = 3).

Available for 88.

Available for 85.

Available for 81.

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APOE Genotyping
Blood samples were genotyped for APOE (gene map locus 19q13.2) using TaqMan Allelic Discrimination technology (Applied Biosystems). Genotypes were obtained for the 2 SNPs that are used to unambiguously define the ε2, ε3, and ε4 alleles (rs7412 and rs429358).

CSF Collection and Analyses
CSF was collected in polypropylene tubes, centrifuged at room temperature for 10 min, the supernatant aliquoted into polypropylene tubes, and frozen at −80°C pending analyses. Mean time from CSF sampling to freezing was below 90 min. Samples were sent on dry ice to the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden, for analyses. CSF Aβ42, total tau (T-tau), and phosphorylated tau (P-tau) concentrations were determined using INNOTEST enzyme-linked immunosorbent assays (Fujirebio). CSF NFL concentrations using a commercial ELISA (UmanDiagnostics, Umeå, Sweden) and YKL-40 concentrations were measured using a commercially available ELISA (R&D Systems). Analyses were performed by board-certified laboratory technicians masked to clinical data. Intra-assay coefficients of variation were 9–13%. We defined Aβ positive as values below 550 pg/mL, which is within conventionally used cut-off values for CSF Aβ42 described in the literature, ranging from 500 to 650 pg/mL (Fagan et al. 2009; Mulder et al. 2010; Niemantsverdriet et al. 2016; Zwan et al. 2016). This threshold also fits the bimodal distribution of the Aβ42 values in the current study (see Supplemental Figure).

Sleep Assessment
Sleep quality was assessed after the second MRI using the PSQI (Buysse et al. 1989) in Norwegian, that is, 3 years after baseline CSF assessment. PSQI is a well-validated self-rated questionnaire that assesses 7 domains of sleep quality (sleep quality, latency, duration, efficiency, problems, medication, and daytime tiredness) in addition to a global score over a 1-month time interval. The minimum score is 0 and maximum score is 21 for each domain, while the global score ranges from 0 to 21.

Dried Blood Spot Analyses
Cholesterol, vitamin D, and docosahexaenoic acid (DHA: C226n3) were assessed at the 2-year follow-up. Blood biomarker levels were monitored in dried blood spots (DBSs) as developed by Vitas (www.vitas.no). The different devices of the collection kit have been carefully selected and tested to provide easy and robust collection of DBSs. The analytical assays are mainly based on different chromatographic techniques. For details, see Walhovd et al. (2014).

Statistics
First, Pearson correlations were run to test the relationship between sleep and a number of confounding variables to decide which should be included as covariates in the following analyses: age, body mass index (BMI), cholesterol, vitamin D, and DHA (C226n3). To test the relationship between CSF biomarkers and sleep, multiple regressions were run with total sleep score as dependent variable and each of the biomarkers T-tau, P-tau, YKL-40, and NFL in turn as predictors, on the form

\[
\text{Sleep} = C + \beta_1 \times \text{Age} + \beta_2 \times \text{Biomarker} + \beta_3 \times \text{Aβ status} + \beta_4 \times (\text{Aβ status} \times \text{Biomarker}).
\]

This way we could test whether each biomarker was differentially related to sleep as a function of Aβ status. Aβ was dichotomized into Aβ+ (<550 pg/mL) and Aβ− (≥550 pg/mL) groups, partly because this is conventional in the literature, and partly because the distribution of Aβ typically is bimodal with a thicker left tail, which also seems to be the case with the current data (see Supplemental Figure). As APOE has been shown to impact the relationship between sleep and neurofibrillary tangle pathology (Lim et al. 2013), additional models were run with APOE status as covariate (presence or absence of an ε4 allele). Post hoc analyses were run with the different PSQI sub-scores as dependent variables. All variables were z-transformed to avoid problems with multi-collinearity.

To test whether effects of biomarkers on sleep could be explained by brain atrophy, we first ran a stepwise multiple regression analysis to identify the optimal linear combination of brain variables to account for the variation in PSQI Global score. In the first step, age was forced into the model. In the second step, SPC in thickness, area and volume in all 33 cortical regions were entered and removed in an iterative stepwise manner. The regions included in the final model were then added as additional covariates in the multiple regression models with the CSF biomarkers, and the β’s were inspected. Reduction in the β’s for the CSF biomarkers in the prediction of sleep problems would be taken as evidence that brain change could account for the sleep—CSF biomarker associations.

The relationship between sleep and clinical and cognitive outcome variables was tested by running partial correlations...
between PSQI Global and the MADRS (Montgomery and Asberg 1979) score at baseline as well as at follow-up, controlling for the effects of age and sex. Further, PSQI Global was correlated with the total score and the number of items recalled from the verbal memory test from the CERAD (The Consortium to Establish a Registry for Alzheimer’s Disease) (Heyman et al. 1990) battery at follow-up, as well as the symmetrized change between baseline and the 2-year follow-up, again controlling for age and sex. Analyses were also run for MADRS and CERAD verbal memory simultaneously to test for the specificity of the relationships.

Results

Relationship Between CSF Biomarkers and Sleep

Total Tau and Phosphorylated Tau
First, the models were run with tau, age, Aβ status, and tau x Aβ interaction as covariates. An interaction with Aβ was found for T-tau (β = 0.63, P = 0.046, R² = 0.076) and P-tau (β = 0.68, P = 0.032, R² = 0.082) with PSQI Global. Post hoc analyses revealed a significantly stronger positive correlation between tau and PSQI Global in the Aβ+ group (total tau: r = 0.42; P-tau: r = 0.45) compared with the Aβ− group (total tau: r = 0.00, P-tau: r = −0.01). The Aβ interactions survived inclusion of APOE status and tau x APOE status interactions for P-tau (β = 0.74, P = 0.043, R² = 0.088), while for T-tau, the slight increase in P-value rendered the interaction significant at a trend level only (β = 0.72, P = 0.066, R² = 0.082). No other variables were significant in these models.

YKL-40
A model with YKL-40, age, Aβ status, and the YKL-40 x Aβ interaction was run. YKL-40 interacted with Aβ in prediction of PSQI Global (β = 0.73, P = 0.026, R² = 0.077). This was due to a stronger relationship in Aβ+ than Aβ− participants (r = 0.33 vs. −0.07). Adding APOE as well as the YKL-40 x APOE interaction increased the YKL-40 x Aβ P-value somewhat for PSQI Global, yielding significance at a trend level only (β = 0.74, P = 0.081, R² = 0.091).

Neurofilament Light
Running the same model as above with NFL as the biomarker of interest, NFL was not significantly related to PSQI Global in the full model including age and Aβ, and did not interact with Aβ (β = −0.13, ns, R² = 0.023).

Effects of Atrophy
A stepwise multiple regression analysis was first run to identify the optimal linear combination of brain variables to account for PSQI Global. In the first step, age was forced into the model. In the second step, SPC in thickness, area and volume in all 33 cortical regions were entered and removed in an iterative step-wise manner. The final model consisted of 7 brain variables (see Table 2), all significantly (P < 0.05) related to PSQI Global. Adjusted R² was 0.30 (P < 0.00005) and all variables except age were significant. A scatterplot illustrating the relationship between PSQI score and area change in caudal anterior cingulate is shown in Figure 2 (right panel). A post hoc analysis showed that there was no interaction between atrophy and

Table 2 Brain variables predicting PSQI

<table>
<thead>
<tr>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>6.24</td>
<td>0.49</td>
<td>12.76</td>
</tr>
<tr>
<td>Age</td>
<td>0.08</td>
<td>0.38</td>
<td>0.02</td>
</tr>
<tr>
<td>Caudal anterior cingulate area thickness</td>
<td>−2.67</td>
<td>0.77</td>
<td>−0.36</td>
</tr>
<tr>
<td>Isthmus cingulate area volume</td>
<td>2.28</td>
<td>0.59</td>
<td>0.42</td>
</tr>
<tr>
<td>Temporal pole thickness</td>
<td>2.00</td>
<td>0.58</td>
<td>0.34</td>
</tr>
<tr>
<td>Insula thickness</td>
<td>−2.21</td>
<td>0.88</td>
<td>−0.27</td>
</tr>
<tr>
<td>Transverse temporal area volume</td>
<td>−1.18</td>
<td>0.52</td>
<td>−0.23</td>
</tr>
<tr>
<td>Inferior parietal area</td>
<td>−3.22</td>
<td>1.08</td>
<td>−0.30</td>
</tr>
<tr>
<td>Parahippocampal area</td>
<td>1.77</td>
<td>0.78</td>
<td>0.23</td>
</tr>
</tbody>
</table>

All variables represent SPC in thickness, area, or volume.

Figure 2. Prediction of sleep problems. Left panel: Scatterplot showing predicted (x-axis) and observed sleep problems (PSQI total score, y-axis) from age, P-tau, Aβ-status, the Aβ x tau interaction and 2 years change in brain structure in 7 different cortical regions. Right panel: Scatterplot illustrating the relationship between sleep problems and change in brain morphometry, in this case % loss of caudal anterior cingulate area over 2 years.
Aβ-status in prediction of PSQI Global. For the detailed results of these analyses, including statistics for all the regions and measures entered, see the Supplementary Information.

After having established the optimal linear combination of brain variables, these were entered into a multiple regression analysis together with age, tau, Aβ-status, and the Aβ × tau interaction to test whether the brain variables could account for the relationship between sleep and the CSF biomarkers. The Aβ × tau interaction survived the inclusion of all the brain variables (β = 0.75, P < 0.05, adjusted R² = 0.37). The results for P-tau were similar, with the interaction with Aβ still significant (β = 0.83, P < 0.01, adjusted R² = 0.39).

The same analyses were run for YKL-40. As for tau, the interaction with Aβ survived introduction of all the brain change variables (β = 0.65, P < 0.05, adjusted R² = 0.36).

Post hoc, these analyses were repeated with sex, BMI, and interval between MRIs in turn as additional covariates. Adding sex or interval did not affect any of the reported relationships. Adding BMI, the Aβ × P-tau interaction was still significant (β = 0.79, P < 0.05, adjusted R² = 0.39), while the P-values for the Aβ × T-tau and Aβ × YKL-40 interactions increased slightly (T-tau: β = 0.64, P = 0.061, adjusted R² = 0.36; YKL-40: β = 0.58, P = 0.069, adjusted R² = 0.35). The contributions from BMI were not significant in any of the models, the changes in β's for the interaction terms were not significant, and the model fits did not improve (T-tau: adjusted R² reduced from 0.37 to 0.36, for YKL-40 from 0.36 to 0.35). Thus, including BMI in the final models was not justified, but we still report that BMI may exert a minor influence on the Aβ-interaction terms.

Relationship to Clinical and Cognitive Outcomes

We tested how depressive symptoms and memory test scores at baseline and 2-year follow-up correlated with sleep problems at the 3-year follow-up. We ran partial correlations between PSQI Global and MADRS at baseline and follow-up, controlling for the effects of age and sex. Significant partial correlations were found at both time points (Baseline: r = 0.56, follow-up r = 0.46, P < 0.00001). Further, we correlated PSQI Global with the total score and the number of items recalled from the verbal memory test from CERAD, again controlling for age and sex, and found significant correlations both for the total score (r = –0.22, P < 0.05) and for the number of items remembered (r = –0.27, P < 0.05). No significant correlations between PSQI at the 3-year follow-up and change in memory score between baseline and the 2-year follow-up were seen, only a trend for number of items recalled (r = –0.18, P = 0.099), indicating that worse longitudinal memory outcome tended to be associated with higher PSQI score.

We reran the memory-PSQI correlations controlling for MADRS scores, and the MADRS–PSQI correlations controlling for memory scores, in both cases also controlling for age and sex. The memory-PSQI correlations were not significant, with a trend only for number of items remembered (r = –0.20, P = 0.07). The MADRS–PSQI correlations were still significant (r = 0.53 and 0.47 for baseline and follow-up, respectively, both P's < 0.00001).

Identification of Confounding Variables

Pearson correlations between PSQI and a number of potential confounding variables are shown in Supplemental Table 1. None of the variables correlated significantly (all P’s > 0.3) with PSQI Global.

Joint Analysis

A final multiple regression analysis was conducted to integrate several tested variables in one comprehensive model. PSQI Global score was used as outcome. As a single measure of atrophy, we used the standardized predicted values from the stepwise regression analysis described earlier (Table 2). P-tau was preferred over T-tau, and the CERAD 10 words number of items recalled from the 2-year follow-up was used as the measure of memory. Further, we included age, MADRS score at 2-year follow-up and YKL-40, as well as Aβ status, the Aβ × P-tau and Aβ × YKL-40 interaction terms. In the initial model, YKL-40 did not contribute significantly. The model was thus re-run without YKL and Aβ × YKL-40. Now, all variables except Aβ positivity and age yielded unique contributions to explain PSQI score (see Table 3 for details). Adjusted R² for this model was 0.53, F = 13.35 (P < 10⁻¹⁰). The predicted values from the model are shown in Figure 2.

Discussion

The results showed that higher CSF levels of tau and YKL-40 predicted poor sleep after 3 years in Aβ positive but cognitively healthy older adults. Further, sleep was predicted from multiple variables, with memory function, depressive symptoms, brain atrophy, P-tau, and Aβ × Ptau interaction all yielding unique contributions. This suggests that sleep is affected by a range of different processes in the brain. Thus, age-related changes in sleep patterns can have multiple causes, which will likely be partly independent, and partly synergistic in explaining sleep disturbances.

Aβ Interacts with Tau and YKL-40 in Predicting Sleep

The relationship between Aβ accumulation and sleep problems has received much attention. Animal studies suggest that Aβ clearance is most efficient during sleep that disturbed sleep will lead to increased Aβ accumulation (Xie et al. 2013) and that Aβ accumulation predicts sleep fragmentation (Roh et al. 2012). In AD, a bidirectional relationship between Aβ and sleep problems may potentially emerge through a number of mechanisms. These include plaque accumulation in the hypothalamus—which contains critical sleep regulating regions such as the ventrolateral preoptic nucleus, and in the periaqueductal gray matter—where Aβ pathology is frequent in the dopaminergic

Table 3 Multiple regression model

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
<td>β</td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>8.34</td>
<td>1.62</td>
<td>5.15</td>
<td>0.00</td>
</tr>
<tr>
<td>Age</td>
<td>–0.31</td>
<td>0.32</td>
<td>–0.08</td>
<td>–0.96</td>
</tr>
<tr>
<td>Memory</td>
<td>–0.35</td>
<td>0.18</td>
<td>–0.08</td>
<td>–1.87</td>
</tr>
<tr>
<td>Depression</td>
<td>0.28</td>
<td>0.08</td>
<td>0.29</td>
<td>3.32</td>
</tr>
<tr>
<td>P-tau</td>
<td>–1.77</td>
<td>0.95</td>
<td>–0.45</td>
<td>–1.87</td>
</tr>
<tr>
<td>Aβ status</td>
<td>–0.33</td>
<td>0.65</td>
<td>–0.04</td>
<td>–0.51</td>
</tr>
<tr>
<td>P-tau × Aβ status</td>
<td>1.72</td>
<td>0.68</td>
<td>0.61</td>
<td>2.52</td>
</tr>
<tr>
<td>Atrophy</td>
<td>1.90</td>
<td>0.31</td>
<td>0.54</td>
<td>6.16</td>
</tr>
</tbody>
</table>

PSQI is the dependent variable, atrophy is the predicted value from the regression analysis as shown in Table 2. P-tau and Aβ are z-transformed. Adjusted R² = 0.53, F = 13.35 (P < 10⁻¹⁰).
wake-active area, as well as more generally through neuronal circuit dysfunctions related to Aβ pathology (Holth et al. 2017). Further, several have reported that sleep problems are associated with accumulation of Aβ-proteins even in healthy older adults (Mander et al. 2015; Brown, Rainey-Smith, Bucks, et al. 2016). For instance, 4 recent studies using amyloid positron emission tomography (PET) found relationships between self-reported sleep parameters and Aβ-accumulation (Spira et al. 2013; Sprecher et al. 2015; Branger et al. 2016; Brown, Rainey-Smith, Villemagne, et al. 2016). Importantly, however, the relationships reported are usually relatively weak, and the exact sleep parameter showing relationship to Aβ varies. Of the studies cited above, significant relationships with Aβ were found for 1 of 6 sleep parameters tested (Brown, Rainey-Smith, Villemagne, et al. 2016), 1 of 4 (Branger et al. 2016), 3 of 7 (Sprecher et al. 2015), and 1–2 (depending on which covariates were used) of 5 (Spira et al. 2013). This suggests that the direct association between self-reported sleep problems and Aβ is not strong. This conclusion warrants efforts to look at other pathways to sleep problems, which may work in synergy with Aβ.

There have been few attempts to test how different biomarkers interact with Aβ in causing sleep disturbances. We found tau to predict poorer sleep better in Aβ positive compared with Aβ negative older adults. This is interesting, as tau accumulation in MTL is one of the first events in the course of AD, likely prior to Aβ accumulation (Braak and Braak 1985), and MTL is critical for many aspects of sleep (Diekelmann and Born 2010; Staresina et al. 2015; Mander et al. 2016). In rodents, MTL tau diminishes expression of hippocampal ripples, causing less temporally synchronized ripple events (Witton et al. 2016) and disrupted network activity during sleep (Menkes-Caspi et al. 2015). Human studies have also reported relationships between tau and sleep patterns (Liguori et al. 2014, 2016; Osorio et al. 2016). In one study, better sleep was associated with less neurofibrillary tangle density at autopsy, attenuating the effect of APOE ε4 (Lim et al. 2013). In this study, no direct effect of sleep was found on plaque load, and sleep modified the APOE effect on tangle density in a manner not statistically mediated by Aβ. In the model used in the present study, sleep was predicted from tau, since tau was observed at an earlier point in time. However, in reality, the causality may go both ways. For instance, long-term sleep deprivation has been shown to lead to memory decline and disruptions of tau processing in transgenic mice (3xTg), with some variations across studies (Rothman et al. 2013; Di Meco et al. 2014).

Compared with the massive research on the relationship between Aβ and sleep problems, less effort has been spent on understanding the effects of tau pathology. The current findings indicate that the combination of Aβ accumulation and tau is related to sleep, in possible vicious causal cycles, leading to the crucial question of whether and how they are redundant, independent or work synergistically in explaining sleep. Tau was more predictive of sleep patterns over 3 years in Aβ positive than Aβ negative older healthy adults. This can be interpreted within a neural reserve model, where sleep problems do not cause or relate to neural damage as indicated by tau levels, unless you already have lesions in the form of accumulated Aβ. Accumulated Aβ is related to increased AD risk, and the results thus indicate that older adults at higher AD risk also are more inclined to sleep problems in the presence of additional brain pathology. As argued by Mander et al. (2017), an important question is whether a relationship between tau and sleep problems is specific to AD or present in other tauopathies. The present study cannot provide a conclusion to this question, but the observation that tau-sleep relationships existed in participants with higher AD-risks only could indicate that this is not a part of normal aging. It has been suggested that dementia-related neuropathologies are related to sleep problems that are different or exaggerated compared with sleep problems seen in normal aging (Mander et al. 2017). As the participants in the current study all have normal cognitive function, it remains to be seen with further follow-ups whether the reported Aβ × tau interactions signify early AD pathological processes or rather aspects of normal aging. Still, this is an important question because it could lead the way to conceptualization of certain sleep problems as an early biomarker for pathological brain decline (Mander et al. 2017). It has also been suggested that tau pathology could cause degeneration of noradrenergic neurons in locus coeruleus, which may play a role in tau induced sleep dysregulation even at the stage of normal aging (Holth et al. 2017). Locus coeruleus typically show abnormal tau phosphorylation prior to cortical tau or Aβ pathology (Braak et al. 2011), and degeneration of this region has then been shown to contribute to Aβ pathology in transgenic mice (Heneka et al. 2006). This constitutes one example of a pathway that links sleep problems, tau and Aβ pathology in a way that will be evident in a tau × Aβ interaction as observed in the present study.

In addition to tau, the neuroinflammation/ astroglial activation marker YKL-40 also interacted with Aβ in predicting sleep. YKL-40 in serum has been related to specific sleep conditions and disturbances (Jafari et al. 2014; Li et al. 2014; Duru et al. 2015; Sun et al. 2015; Jafari and Mohsenin 2016). A link between microglia and Aβ accumulation has been established, in which Aβ represents prime stimuli for inflammation (Akiyama et al. 2000; Lee et al. 2008). CSF levels of YKL-40 (Craig-Schapiro et al. 2010) increase with age (Schuitenmaker et al. 2012) and in AD (Craig-Schapiro et al. 2010; Antonell et al. 2014; Rosen et al. 2014; Janelidze et al. 2016) (but see Mattsson et al. (2011) for a study finding no effect on AD), and are related to brain atrophy (Alcolea et al. 2015; Gispert et al. 2016). Put together, these factors can explain why YKL-40 may play a crucial role in age-related sleep disturbances, and the observed interaction between Aβ and YKL-40. It must be noted, however, that when all variables were included in the multiple regression model, YKL-40 no longer uniquely predicted sleep.

We and others have recently shown that NFL is related to hippocampal atrophy (Idland et al. 2016; Zetterberg et al. 2016), but NFL did not interact with Aβ in predicting sleep problems in the current data. Further studies are necessary to establish whether NFL is related to sleep characteristics.

Multiple Variables Predict Sleep

Importantly, multiple variables uniquely predicted sleep patterns. In addition to the tau × Aβ interaction, brain atrophy, memory function and depressive symptoms scores uniquely contributed to explain variance in the global sleep variable. This suggests that variation in sleep index a range of different processes. In this model, sleep was a dependent variable, predicted, for example, from memory, rather than the other way around. Of course, this should not be taken as evidence that the mechanisms work only one way, as there for instance are obvious effects of sleep on memory function (Rasch and Born 2007; Stickgold and Walker 2013; Klinzing et al. 2016). Rather, this result indicate that memory and sleep are related also if a range of different biomarkers are accounted for. This is in accordance with previous findings that memory and sleep are related on a mechanistic level and not just through shared
variance with other important variables. Also, sleep was predicted from tau levels, but experimental rodent studies suggest possible pathways from sleep deprivation to tau (Rothman et al. 2013; Di Meco et al. 2014) and Aβ accumulation (Musiek and Holtzman 2016). Still, the results underscore that sleep may be an important variable related to brain health and cognitive function in older adults at increased risk of Alzheimer’s disease according to their status as Aβ positive.

Limitations

There are several limitations to the present study. First, although sleep was measured by a well-validated and much used inventory, it is still a self-report measure. The strength of this approach is that sleep is measured in the participants’ natural environment, increasing its ecological validity. Complementary evidence from actigraphy would strengthen the conclusions. Further, sleep disorders were not screened out at baseline or follow-up. Second, participants with cognitive decline were screened out. This may have biased the sample, as for instance older adults with high rates of atrophy, Aβ accumulation and sleep problems also would be more likely to be excluded, thus possibly reducing the observed interrelations between the variables of interest. Still, this approach allowed us to study the relationships among multiple variables in the very important group of cognitively well-functioning older adults with different Alzheimer risk profiles, representing the most promising time window for possible intervention. Also, although CSF measures yield highly accurate indexes of total biomarker levels, we do not get information about where in the brain accumulation is largest, which is a benefit of, for example, amyloid PET. Finally, the analyses of brain structural change were performed mainly to allow us to test whether the CSF biomarkers were predictive of sleep problems independently of atrophy. Thus, we used a stepwise regression analysis approach to identify the optimal linear combination of structural brain variables accounting for variation in sleep. This constituted the most stringent test of the power of the CSF biomarkers to predict sleep problems independently of structural brain changes. However, due to covariance between brain structural variables, this means that each of the resulting brain regions and measures are not necessarily among the most important for sleep problems or mechanistically involved in sleep. Even though some of the regions correspond well to what has been found in previous research, for example, the caudal anterior cingulate, insula and transverse temporal cortex (Sexton et al. 2014), the regions included in Table 2 should therefore be interpreted on this background.

Conclusion

The present results demonstrate that Aβ interacts with tau and YKL-40 in predicting sleep problems, in that poor sleep was predicted by higher levels of tau and YKL-40 in Aβ positive participants only. Further, lower memory function, higher depression scores and more brain atrophy also uniquely predicted poorer sleep, clearly suggesting that sleep is related to a range of different processes in the aging brain.

Supplementary Material

Supplementary material is available at Cerebral Cortex online.

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Notes

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References


Klingenberg et al. 2014. Association of serum YKL-40 with the prediction of Sleep. Fjell et al. 9.


