SLEEPJ, 2021, 1–10

doi: 10.1093/sleep/zsaa195
Advance Access Publication Date: 21 September 2020
Original Article

ORIGINAL ARTICLE

Alzheimer's disease neuropathology in the hippocampus and brainstem of people with obstructive sleep apnea

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Abstract

Obstructive sleep apnea (OSA) involves intermittent cessations of breathing during sleep. People with OSA can experience memory deficits and have reduced hippocampal volume; these features are also characteristic of Alzheimer's disease (AD), where they are accompanied by neurofibrillary tangles (NFTs) and amyloid beta (Aβ) plaques in the hippocampus and brainstem. We have recently shown reduced hippocampal volume to be related to OSA severity, and although OSA may be a risk factor for AD, the hippocampus and brainstems of clinically verified OSA cases have not yet been examined for NFTs and Aβ plaques. The present study used quantitative immunohistochemistry to investigate *postmortem* hippocampi of 34 people with OSA (18 females, 16 males; mean age 67 years) and brainstems of 24 people with OSA for the presence of NFTs and Aβ plaques. OSA severity was a significant predictor of Aβ plaque burden in the hippocampus after controlling for age, sex, body mass index (BMI), and continuous positive airway pressure (CPAP) use. OSA severity also predicted NFT burden in the hippocampus, but not after controlling for age. Although 71% of brainstems contained NFTs and 21% contained Aβ plaques, their burdens were not correlated with OSA severity. These results indicate that OSA accounts for some of the "cognitively normal" individuals who have been found to have substantial Aβ burdens, and are currently considered to be at a prodromal stage of AD.

Statement of Significance

Evidence links obstructive sleep apnea (OSA) and Alzheimer's disease (AD), yet the underlying neuropathology of AD has not been investigated in OSA. The present study analyzed autopsy brain tissue from confirmed OSA cases for amyloid beta (Aβ) and tau, hallmark proteins of AD. As OSA severity increased, the burden of Aβ increased in the hippocampus, even after controlling for age, sex, body mass index (BMI), and continuous positive airway pressure (CPAP) use. Age, not OSA severity, was the strongest predictor of tau pathology. The majority of brainstems contained tau, while a smaller proportion had Aβ plaques. Although the sample size is limited, these findings strengthen the association between OSA and AD.

Key words: amyloid beta; continuous positive airway pressure; Alzheimer's disease; neurofibrillary tangles; tau

Submitted: 29 March, 2020; **Revised:** 10 September, 2020

Introduction

Obstructive sleep apnea (OSA) involves intermittent cessations of breathing during sleep, leading to transient arterial oxygen desaturations and arousals. Overall estimates of prevalence range from 9% to 38% in adults and increase with age [1, 2]. Continuous positive airway pressure (CPAP) during sleep provides effective symptomatic treatment [3]. People with OSA frequently display deficits in attention, memory, and executive functioning [4]. While these deficits improve with CPAP treatment, the extent of recovery is less complete in people with moderate–severe OSA [5], suggesting that severe OSA may permanently injure the brain.

Alzheimer's disease (AD) is characterized by a progressive deterioration of memory, executive dysfunction, and reduced volume of the hippocampus and parahippocampal gyrus [6–8]. The hippocampal atrophy is accompanied by an accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles (NFTs), which accumulate in the rostral part of the parahippocampal gyrus and then spread to the adjacent hippocampus, and other cortical regions become involved [9–11]. The primary causative agent(s) of AD is (are) unknown; however, the pattern of hippocampal pathology is well characterized, although there remains some debate as to which neuropathological feature occurs first, Aβ or NFTs [12, 13].

OSA is an independent risk factor for the development of dementia in cognitively normal elderly persons; females [14] and males [15] with OSA have a 1.70 times and 1.44 times increased risk of developing dementia in the next 5 years, respectively. More recently, severe OSA was associated with a 2.35 times increased risk of all-cause dementia and a 1.66 times increased risk of AD specifically [16]. A recent review illustrates numerous shared pathogenic pathways between OSA and AD that could account for this increased risk [17].

Like in AD, people with severe OSA exhibit hippocampal atrophy [5, 18, 19], but it is not known whether the atrophy is accompanied by the presence of Aβ plaques and NFTs. It is notable that studies using chronic intermittent hypoxia to model OSA in rats, as well as studies of AD-transgenic mice, have demonstrated that intermittent hypoxia leads to a significant increase in the cerebral and hippocampal burdens of Aβ [20, 21]. Further, the initial stage of NFT development, tau hyperphosphorylation, is promoted by hypoxia [22, 23]. Together these animal studies show that intermittent hypoxia is capable of precipitating neuropathological changes that resemble those seen in AD. Additionally, people with OSA have decreased CSF Aβ and increased CSF tau compared to control subjects [24], a pattern also observed in AD [25]. Positron emission tomography (PET) imaging suggests that people with OSA have a small but significantly increased global amyloid burden [26] and specific increases in the temporal lobe and cingulate gyrus [27] compared to controls. No difference has been found for tau-PET imaging in people with OSA [26]. A recent study found increased tau-PET in people with "witnessed apneas" compared to those without [28]; however, "witnessed apneas" are not an objective or clinical measure of OSA. In addition, tau-PET ligands lack specificity, particularly first-generation ligands which were used in both of the above studies [29]. Additionally, amyloid detected by PET imaging accumulates faster in people with OSA compared to those without [30]; however, this study did not measure the severity of OSA. A study including apnea-hypopnea index (AHI) as an OSA severity measure did not find an increase in amyloid burden related to OSA severity over a 2-year follow-up [31]. A more recent PET study found a significantly higher amyloid burden in the brains of people with severe OSA compared to those with mild/moderate or no OSA [32]. It remains to be seen whether severity of OSA increases the incidence of Aβ plaques and NFTs in the hippocampus, the region most affected by AD pathology.

In AD, studies have revealed the presence of hyperphosphorylated tau and NFTs in the brainstems of people with AD, particularly in the locus ceruleus (LC) and dorsal raphe nucleus [33, 34]. There are suggestions that hyperphosphorylated tau and NFTs may occur early in the pathogenesis of AD, prior to the appearance of these features in the hippocampus [12]. However, these neurodegenerative changes have not been studied in as much detail as the hippocampal pathology, and the specific spatiotemporal pattern of accumulation in the brainstem is yet to be fully elucidated. Interestingly, the LC and dorsal raphe nucleus both play a role in maintaining wakefulness; the neurons in both brainstem nuclei are active during wakefulness and inactive during slow wave sleep and rapid eye movement (REM) sleep [35]. Wake-activated neurons of the LC are impaired or lost entirely in animals exposed to chronic intermittent hypoxia or sleep fragmentation, which are both models of OSA [36, 37].

Only one study [38] has examined the human brain for evidence of AD neuropathology associated with sleep and oxygenation variables. The study investigated autopsied brain tissue from 167 men who had died within 10 years of an overnight polysomnography, and found that the extent of neuronal loss from the LC is associated with the extent of arterial deoxygenation during sleep. While the LC was not examined for the presence of Aβ plaques or NFTs, tissue from four neocortical regions was examined, and the lack of significant pathology led the authors to conclude that sleep and oxygenation fluctuations are unlikely to contribute to AD pathogenesis. However, Gelber and colleagues [38] did not examine the hippocampus or parahippocampal gyrus, where the first neuropathological changes of AD are typically identified, nor did they investigate whether NFTs are present in brainstem nuclei. Consequently, any AD-like neuropathological changes occurring in these brain regions would have been undetected.

The present study examined autopsied brain tissue from people with clinically verified OSA, to determine whether OSA severity is associated with an increased burden of Aβ and NFTs. The regions examined were the rostral pons at the level of the LC, and the hippocampus, including the parahippocampal gyrus and adjacent cerebral cortex. Age, sex, body mass index (BMI), apolipoprotein E (APOE) status, and CPAP use were investigated for their potential to contribute to any observed effects.

Methods

This project was approved by the National Bioethics Committee, Iceland (reference 09-087-CM) and the RMIT Human Research Ethics Committee, Australia (reference ASEHAPP 71-16).

Study sample

The present study sample consisted of autopsy brain tissue from 34 Icelandic persons (mean age 67.0 ± 11.1 years) with OSA described previously [19]. These 34 people (18 females with mean age 67.3 ± 12.5 and 16 males with mean age 66.7 ± 9.7 years) had

autopsy samples taken from the medial part of the temporal lobe, including the hippocampus and parahippocampal gyrus. A subset of 22 subjects had autopsy tissue taken from the brainstem at the level of the rostral pons, including the LC. Brainstem tissue was available for two additional people who did not have hippocampal tissue available. In total there were 34 people with hippocampal tissue, 24 with brainstem tissue and 22 with both hippocampal and brainstem tissue. Although subjects were excluded from this study if they had been diagnosed with dementia, micrographs from a person with AD are included for comparative purposes only.

OSA diagnoses were always based on whole-night sleep studies and oxygen desaturation index (ODI) with a 4% drop from baseline was used as the measure of OSA severity, as previously described [19]. All subjects had predominantly obstructive OSA and no minimum ODI was required for inclusion in the study. To investigate the frequency of different types of neuropathology as a function of OSA severity, the sample was split at the median ODI value of 18.6. Those with an ODI less than 18.6 were considered for the purpose of this study to have mild–moderate OSA ("lower ODI") and those with an ODI greater than 18.6 were considered to have moderate–severe OSA ("higher ODI"). A median split was used, rather than the three clinical categories for OSA severity, in order to maximize statistical power.

Among the 34 subjects, 18 were known to have regularly used CPAP until they died (self-reported to the physician or observed by hospital staff). Of the remainder, three were known to have never used CPAP while 11 were not using CPAP at the time of death but may or may not have used CPAP for some period of time between diagnosis and death. For the purposes of this study, only those known to have regularly used CPAP were included in the "CPAP users" group. All other subjects were considered to be "CPAP nonusers." Subjects who underwent surgical treatments for OSA were excluded from the study.

Tissue processing and immunohistochemistry

Tissue processing and immunohistochemistry was performed as previously described [19] during 2014 and 2015. Blocks from the medial part of the temporal lobe (including rostral hippocampus and parahippocampal gyrus) and rostral pons (including LC) were used in the present study.

The immunohistochemistry protocol was modified from that previously described [19]. Briefly, sections to be stained for Aβ underwent antigen retrieval in which sections were incubated in 99% formic acid for 1 hour. Sections to be stained for tau did not require antigen retrieval. Primary antibodies for anti-β-amyloid (monoclonal clone 6F/3D, DAKO, M0872) diluted at 1:100 or anti-tau (polyclonal, Sigma, T6402) diluted at 1:500 were incubated overnight at room temperature. Following this, sections were incubated with appropriate secondary antibodies (Aβ sections, donkey anti-mouse from Merck Millipore AP192B; Tau sections, donkey anti-rabbit from GE Healthcare RPN 1004) diluted at 1:300, followed by streptavidin-biotinylated horseradish peroxidase (GE Healthcare, RPN1051) diluted at 1:300, then diaminobenzidine-nickel sulphate as the chromogen.

All brain sections were processed in an identical manner. When classifying the brain tissue for burden of Aβ plaques and NFTs the examiner (J.E.O.) was blinded to OSA severity and usage of CPAP. The second examiner (S.R.R.) was also blinded to the OSA severity and CPAP use and confirmed the classification of the Aβ plaques and NFTs. For each brain, two hippocampus sections were analyzed for each antibody. The classifications were based on the highest stage or phase reached in the two sections (see following sections). For brainstem sections, one section was analyzed for each brain, due to the limited availability of tissue.

Classification of hippocampal NFT burden

Tau was identified by immunopositive staining for the anti-tau antibody, evident as NFTs, neuropil threads, and neuritic plaques. The location and density of NFTs was used for classification in the hippocampus in accordance with previously defined stages for AD pathogenesis [10]. If no NFTs could be seen in the medial part of the temporal lobe, the section was classified as Stage 0. If NFTs were limited to the transentorhinal region (between the parahippocampal gyrus and the occipitotemporal gyrus), then the section was classified as Stage 1. If NFTs could also be seen in the entorhinal cortex and CA1/CA2 of the hippocampus, then they were classified as Stage 2. When NFTs were present in CA3/CA4 and the occipitotemporal gyrus, they were classified as Stage 3. Where a substantial increase in the density of NFTs was present in the areas affected in Stage 3, they were classified as Stage 4 (Supplementary Figure 1). By convention, Stage 4 is defined using both hippocampal and neocortical tissue; therefore, our Stage 4 is not directly comparable to Stage 4 as defined by Braak and colleagues [10]. However, there are significant hippocampal differences between Stage 3 and 4, and our Stage 4 was based on these differences alone.

Classification of hippocampal Aβ plaque burden

Aβ plaques were staged according to the criteria described by Thal and colleagues for AD pathogenesis [9]. If no plaques could be seen, then the section was given a classification of Phase 0. Brains were classified as Phase 1 if Aβ plaques were limited to the parahippocampal gyrus and/or the occipitotemporal gyrus. When plaques were present in the subiculum and CA1 as well as in the parahippocampal and/or occipitotemporal gyrus, the brain was classified as Phase 2. If plaques extended into CA2/CA3 and the molecular layer of the dentate gyrus, then the brain was classified as Phase 3. If plaques were also present in the hilus and throughout CA4, then the brain was classified as Phase 4 (Supplementary Figure 2).

Classification of pathology in brainstem

There is no established classification system for NFT or Aβ burden in the brainstem. Aβ is rarely found in the brainstem in early AD but was investigated here because the effect of OSA on brainstem Aβ is unknown. Consequently, the present study conducted simple counts of Aβ plaques and NFTs in the brainstem sections. The present study identified the locations of NFTs and any other deposits of tau that could be identified. "Any tau" included NFTs, neuropil threads, and/or neuritic plaques that were immunoreactive for the anti-tau antibody.

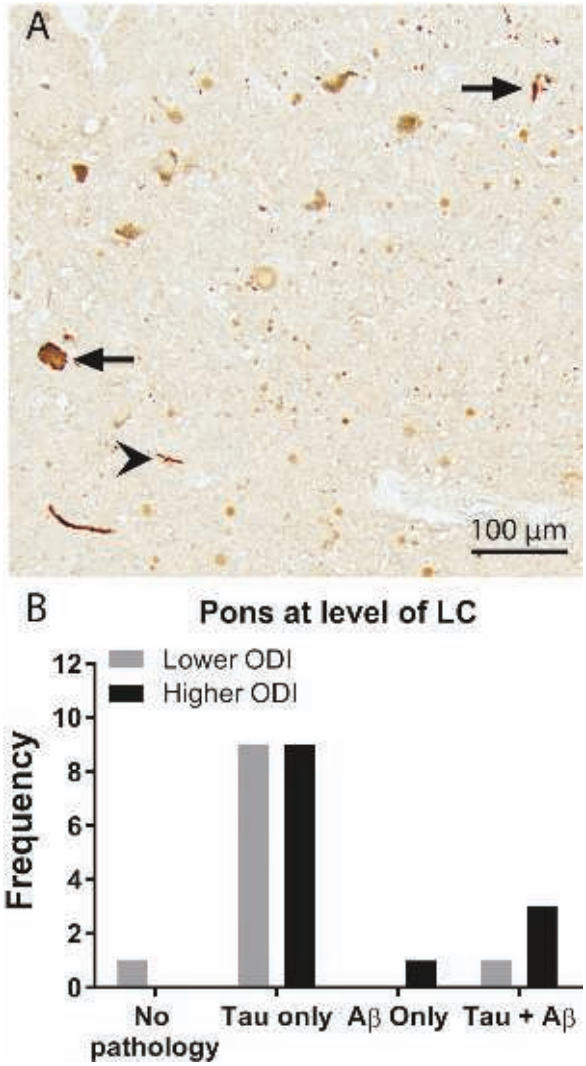

Figure 1. Micrograph of NFTs (arrow) and neuropil threads (arrowhead) in the LC (A). Frequency of pathology in the rostral pons at the level of LC (B), in the lower ODI group (ODI < 18.6; gray bars) and in the higher ODI group (ODI > 18.6; black bars).

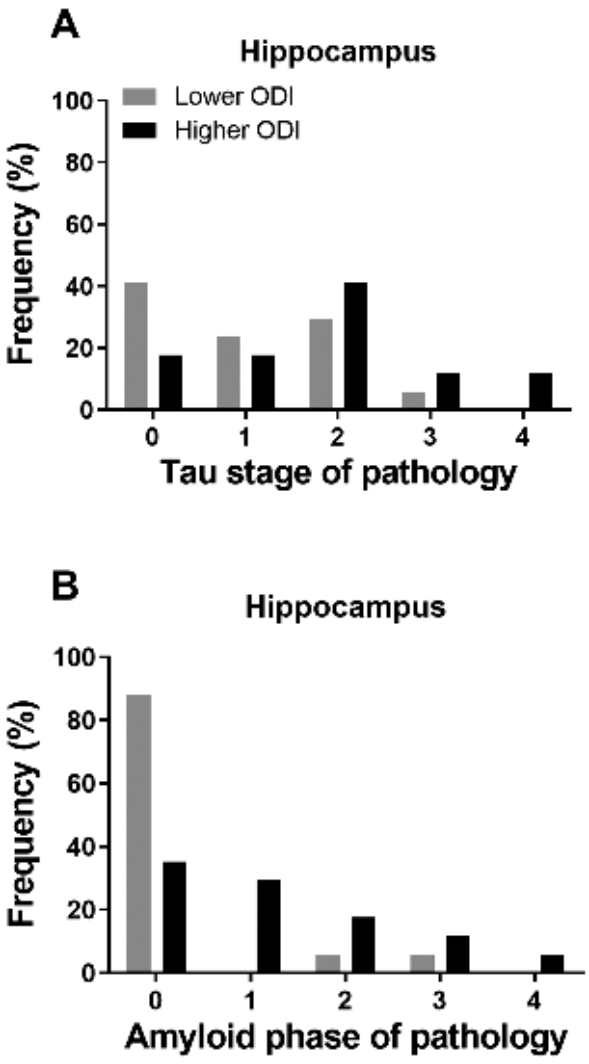

Figure 2. The frequency of tau stage of pathology (A) and Aβ phase of pathology (B) seen in the lower ODI group (ODI < 18.6; gray bars) and the higher ODI group (ODI > 18.6; black bars).

APOE genotyping

APOE genotype was determined with the Sanger sequencing method [39]. Brain tissue was sectioned as above and then collected into Eppendorf tubes. The samples were dewaxed, followed by DNA isolation and amplification using PCR. The two oligonucleotides used for DNA amplification, both from TAG (Copenhagen), were:

APOE sequence F5′ GCGGACATGGAGGACGTG-3′
APOE sequence R5′ CCCCGGCCTGGTACACTG-3′

After successful DNA isolation and amplification samples were sequenced using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) to determine the amino acid residues at 112 and 158 which differ by ApoE genotype [40].

Statistical methods

The variable ODI was found to be non-normally distributed (Supplementary Figure 3), a log transformation was performed resulting in a normal distribution, and therefore analyses were performed with log ODI. All statistical analysis was performed using the IBM SPSS, except for the Fisher's Exact Test for independence which was performed using an online calculator that allowed 2 × 4 and 3 × 3 contingency table calculations (http://vassarstats.net). Independent samples *t*-tests were used to compare CPAP and no CPAP groups on descriptive variables. For the hippocampus, ordinal regression was used to analyze the association between log ODI and tau and amyloid pathology after controlling for the additional variables of age, CPAP use, sex, and BMI.

Results

Descriptive statistics

Descriptive statistics for the brainstem and hippocampus samples can be seen in Table 1. Within each sample, correlations were performed between age, BMI, time from diagnosis to death, and ODI. In the brainstem sample no significant correlations were found between any of the descriptive variables. In the hippocampus sample there was a significant

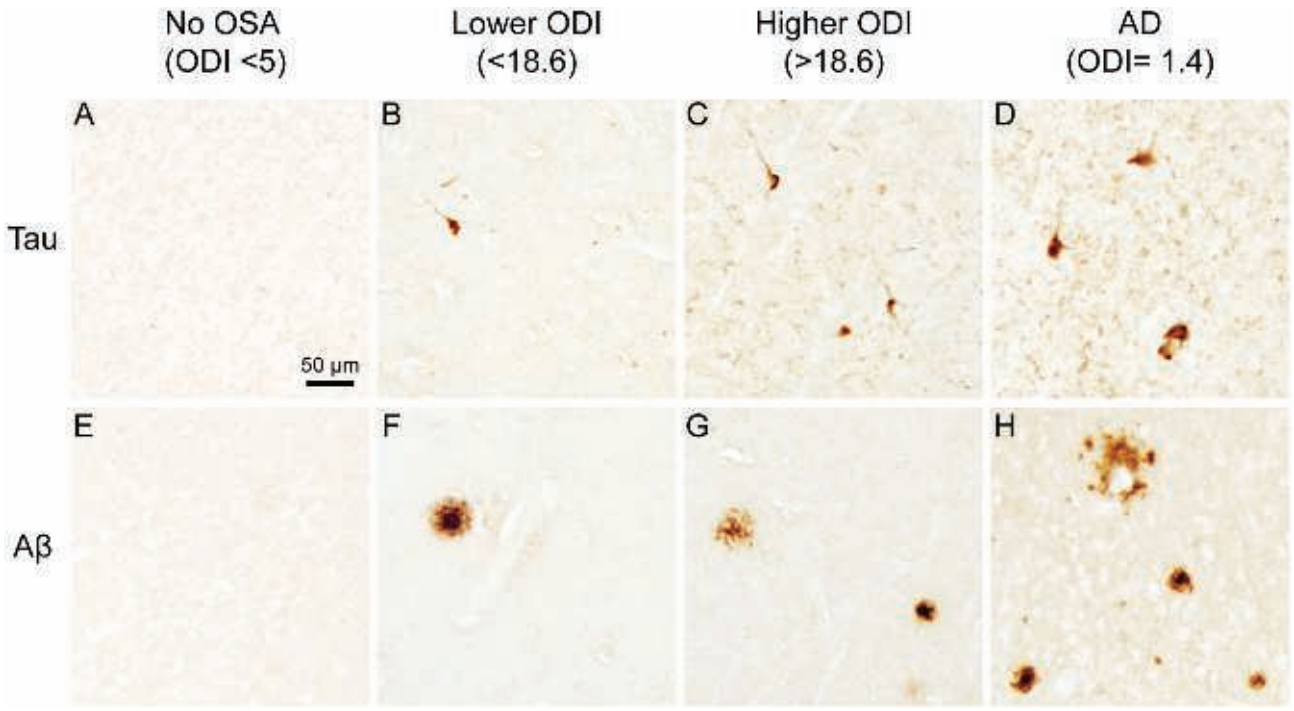

Figure 3. Micrographs of hippocampus sections from people with OSA. A person with no OSA (A, E), lower ODI (B, F), higher ODI (C, G), or AD (D, H). Tau staining indicates NFT (A–D) and Aβ staining indicates amyloid plaques (E–H).

Table 1. Descriptive statistics of brainstem and hippocampus samples with the hippocampus sample, stratified by CPAP use

	Brainstem sample	Hippocampus sample	CPAP users	CPAP nonusers	Significance CPAP users vs. nonusers
n	24	34	18	16	
Gender	F = 14, M = 11	F = 18, M = 16	F = 12, M = 6	F = 6, M = 10	
Age at death (years)	68.3 ± 11.1	67.0 ± 11.1	69.9 ± 11.1	63.8 ± 10.5	$p = 0.111$
BMI kg/m^2	30.7 ± 6.4 *n* = 21	29.5 ± 6.2 *n* = 29	32.6 ± 5.8 *n* = 16	25.7 ± 4.4 *n* = 13	$p = 0.002$*
Time from OSA diagnosis to death (years)	6.6 ± 5.4	7.3 ± 5.8	7.1 ± 6.2	7.5 ± 5.7	$p = 0.815$
ODI (events/h)	26.6 ± 22.6	25.5 ± 21.3	32.0 ± 18.8	18.2 ± 22.0	$p = 0.057$

Mean ± standard deviation.
*$p < 0.05$ CPAP users compared to CPAP nonusers.

correlation between age and ODI ($r = 0.416$, $p = 0.014$). No other correlations were found between these variables. When the hippocampus sample was stratified into CPAP users and CPAP nonusers, no significant difference was found between age, time from diagnosis to death, or ODI. However, BMI was significantly higher for CPAP users compared to CPAP nonusers ($t(27) = 3.5$, $p = 0.002$) and they also had a tendency to higher ODI ($p = 0.057$). No difference in age was found between the lower and higher ODI groups for the brainstem sample (67.6 ± 9.8 vs. 68.9 ± 12.5, respectively) or the hippocampus sample (65.4 ± 10.7 vs. 68.7 ± 11.5). No significant differences were found between males and females for any of the descriptive or neuropathological variables.

APOE genotype

It is well established that the ε4 allele of the APOE gene, which codes for apolipoprotein E, is a genetic risk factor for AD. Despite a recent meta-analysis concluding that there is no increased risk of OSA for APOE-ε4 carriers [41], it is important to know whether APOE genotype is associated with different development of Aβ plaques and NFTs in OSA. APOE genotype was determined for 26 of the 34 subjects; for the remaining eight, tissue processing at autopsy caused irreversible DNA damage that prevented isolation of DNA and therefore genotyping. Of the genotypes that were determined, most people (22/26) were ε3/ε3, three people were ε3/ε4, and one was ε4/ε4. Fisher's Exact Tests were performed between the distribution of ApoE4 allele (+, −, or unknown) and the presence or absence of amyloid. The results found no significant difference in the distribution of ApoE4 allele for those with amyloid compared to those without amyloid in the hippocampus ($p = 0.14$). No significant difference was found for the same analysis for the presence or absence of tau pathology ($p = 0.186$). Due to the amount of missing data and similarity of distribution between those with and without pathology, this variable was not included in the statistical analysis.

Presence of AD pathology in the brainstem

Of the 24 brains with sections of pons that included the LC, five contained Aβ plaques and 22 contained tau. Due to the low number of brains containing any Aβ plaques (n = 1 and 4, for lower and higher ODI, respectively), no further investigation was conducted; however, the location of NFTs was further investigated. NFTs and neuropil threads were seen most frequently in the LC (Figure 1A) and dorsal raphe nucleus with some present in other parts of the pontine reticular formation, pontine nuclei, and mesencephalic nucleus of the trigeminal (Table 2).

Relationship between AD pathology in the brainstem and OSA severity

The frequency of no pathology, tau-only pathology, Aβ-only pathology, and tau + Aβ pathology can be seen in Figure 1B, separated into those with lower and higher ODI scores. The lower and higher ODI groups had similar numbers of people with each type of pathology. The percentage of brains containing any tau is similar for both lower and higher ODI (Table 3). The percentage of brains containing any Aβ is higher for those with higher ODI; however, only five brains contained Aβ. A Fisher's Exact Test for independence found that there was no significant difference between the distribution of pathology depending on OSA severity (n = 24, p = 0.673). Further, no significant correlations were found between log ODI and the number of NFTs in the brainstem overall, r^2 = 0.053, p = 0.289, or the number of NFTs in the LC, r^2 = 0.003, p = 0.789. However, the small number of brainstems with NFTs (16) prevented us from performing regression analyses to control for age, sex, CPAP use, and BMI, so we cannot rule out the possibility that these factors are obscuring a relationship between ODI and NFT.

Relationship between AD pathology in the hippocampus and OSA severity

Hippocampal sections were analyzed from 34 brains. Of these, 26 had Aβ plaques and/or NFTs; 13 brains had NFTs only, while two brains had Aβ plaques only. Both Aβ plaques and NFTs were observed in 11 of the 34 brains. The distributions of Aβ plaques and NFTs were analyzed, and the brains were then staged for tau and Aβ, according to the criteria used for staging these neuropathological features in AD. Brains from people with lower ODI more frequently had earlier stages of tau pathology and no amyloid pathology, whereas those with higher ODI more frequently had later stages of tau pathology and amyloid pathology (Figure 2A and B). The percentage of brains with pathology can be seen in Table 3 for those in the lower and higher ODI groups. In the lower ODI group, a higher percentage of brains had no pathology compared to the higher ODI group. The higher ODI group had a higher percentage of brains with both NFT and Aβ pathology, as well as more with any NFTs and any Aβ pathology.

A Fisher's Exact Test for independence found a significant difference between the distribution of pathology for lower and higher ODI (n = 34, p = 0.006). This relationship is evident in the micrographs which show no tau or Aβ immunoreactivity in a brain with no OSA (Figure 3A and E), a few NFTs or plaques in a brain with lower ODI (Figure 3B and F), and more NFTs as well as neuropil threads or plaques in the brain of a person with higher ODI (Figure 3C and G). Figure 3 includes images from a person with AD dementia for comparison. Some of the people with severe OSA resembled AD dementia pathology in the extent of their NFT and Aβ plaque burden (Figure 3D and H).

Relationship between NFT burden in the hippocampus and OSA severity

In the hippocampus sections, NFTs were observed in 24 of the 34 brains. Of these 24, 7 were at Stage 1, 12 were at Stage 2, 3 were at Stage 3, and 2 were at Stage 4 (Figure 2A). Ordinal regression analysis was performed with NFT stage as the dependent variable (Table 4). Log ODI was a significant predictor of NFT stage when it was the only predictor in the model (p = 0.02). However, when age is included as a predictor in the model, NFT stage is no longer significant (p = 0.262) and age is a significant predictor (p = 0.005). Age remains the only significant predictor when CPAP use, sex, and BMI are also added to the model (p = 0.002).

Relationship between Aβ burden in the hippocampus and OSA severity

In the hippocampus sections, Aβ plaques were observed in 13 of the 34 brains. Of these, five were classified as Phase 1, four as Phase 2, three as Phase 3, and one as Phase 4 (Figure 2B). Ordinal regression analysis found that log ODI was a significant predictor of Aβ phase (p = 0.02) and remained the only significant predictor when age, CPAP use, sex, and BMI were also considered in the model (p = 0.029) (Table 4).

Relationship between AD pathology in the hippocampus and the brainstem

In 22 brains, autopsy samples were available from both the hippocampus and from the brainstem at the level of the pons. Of these, 16 brains had tau in both sites, 4 had tau only in the brainstem, no brains had tau only in the hippocampus, and 2 had tau in neither location. Of the brains with brainstem tau, 15/16 had tau specifically in the LC. Aβ plaque deposition was less consistent. Of the 22 brains that had samples from both locations, 2 had Aβ plaques in both locations, 7 had Aβ plaques in the hippocampus only, 2 had Aβ plaques in the brainstem only, and 11 had no Aβ plaques. Fisher's Exact Tests for independence found no significant relationship between the distributions of pathology in the hippocampus and brainstem for tau (n = 22, p = 0.065) or

Table 2. Percentage (n) of brains with any tau pathology, neuropil threads, or NFTs in different nuclei of the pons at the level of the LC, n = 24

	Locus ceruleus	Dorsal raphe nucleus	Reticular formation	Pontine nuclei	Mesencephalic nucleus
Any tau	87.5% (21)	70.8% (17)	50% (12)	25% (6)	20.8% (5)
Neuropil threads	75% (18)	66.7% (16)	41.7% (10)	20.8% (5)	20.8% (5)
NFTs	70.8% (17)	66.7% (16)	33.3% (8)	16.7% (4)	16.7% (4)

Table 3. Percentage (*n*) of subjects in the lower and higher ODI groups with tau and/or Aβ pathology in the pons at the level of the LC

	Brainstem			Hippocampus		
Pathology	Total sample (*n* = 24)	Lower ODI (*n* = 11)	Higher ODI (*n* = 13)	Total sample (*n* = 34)	Lower ODI (*n* = 17)	Higher ODI (*n* = 17)
No pathology	4% (1)	9% (1)	0% (0)	24% (8)	35% (6)	12% (2)
Tau only	75% (18)	82% (9)	69% (9)	38% (13)	53% (9)	24% (4)
Aβ plaques only	4% (1)	0% (0)	8% (1)	6% (2)	6% (1)	6% (1)
Tau and Aβ plaques	17% (4)	8% (1)	23% (3)	32% (11)	6% (1)	59% (10)
Any tau	92% (22)	91% (10)	92% (12)	71% (24)	59% (10)	82% (14)
Any Aβ plaques	21% (5)	9% (1)	31% (4)	38% (13)	12% (2)	65% (11)

Table 4. Ordinal regression analysis in hippocampus

Dependent variable	Predictor/s	Estimate	*P*-value
NFT stage	Log ODI	2.05	0.020*
NFT stage	Log ODI	1.66	0.176
	Age	0.17	0.002*
	Sex	−0.65	0.415
	BMI	−0.07	0.346
	CPAP use	0.57	0.593
Aβ phase	Log ODI	2.64	0.020*
Aβ phase	Log ODI	3.21	0.029*
	Age	0.03	0.568
	Sex	−0.11	0.897
	BMI	0.05	0.534
	CPAP use	−1.28	0.283

*$p < 0.05$.

Aβ ($n = 22$, $p = 0.550$), suggesting that no direct relationship exists between the presence of these neuropathological features in the two regions. However, the small sample size is a limitation, and given the trend for a significant relationship for tau pathology, this result should be viewed with caution until confirmed in a larger sample.

Discussion

The present study investigated the extent of AD-like neuropathology in autopsy tissue from the hippocampus and brainstems of 34 Icelandic people with clinically verified OSA. Our results show that increased OSA severity tends to be associated with an increased Aβ burden, but not an increased NFT burden in the hippocampus. While there was an association between NFT stage and OSA severity, the relationship did not withstand controlling for age, and age was found to be the only significant predictor of NFT burden. CPAP use did not affect these relationships. No evidence of an association was found in the brainstem. APOE genotypes were distributed as expected for the general population and are not likely to have affected the results. These findings add to our previous findings of reduced hippocampal volume and demyelination associated with increasing OSA severity in the same brain samples [19].

In the present study, 70% of OSA cases had NFTs and 38% had Aβ plaques in their hippocampus, which is broadly comparable to estimates made by Braak and colleagues [42] that approximately 95% of elderly individuals had some degree of NFT burden and 35% had some degree of Aβ burden. Those estimates were derived from a population with a narrower age range (61–70 years) than in the current study (41.7–89 years), which may account for the lower percentage of NFTs found here. Neither the OSA status nor the cognitive status of the subjects were recorded by the Braak study; therefore, their estimates are likely to include people with OSA and with clinical symptoms of AD. Like the present study, Braak and colleagues [42] reported that NFTs are more frequently observed than Aβ plaques in early stages of AD, suggesting that NFT development may precede Aβ accumulation. The present study found that Aβ was rarely present by itself in the hippocampus, whereas NFTs frequently were. Furthermore, in brains with a low NFT or Aβ burden, pathology was consistently limited to the region near the collateral sulcus, whereas in brains with heavier burdens of pathology, the distribution closely resembled those reported for AD [9, 10]. This pattern suggests that the spatiotemporal spread of pathogenesis is identical for AD and OSA.

While some people in the present study may have had mild cognitive impairment or undiagnosed AD, none had symptoms that were salient enough to attract a diagnosis, even though some had a density of NFTs and Aβ plaques that were sufficiently high to qualify as AD. Furthermore, some people with mild OSA had many Aβ plaques and/or NFT, whereas some people with severe OSA had little or no neuropathology. Finally, one person diagnosed with AD (not included in analysis) was confirmed not to have had OSA (Figure 3G and H). Together these observations indicate that while OSA tends to increase the burden of Aβ neuropathology, *the presence of OSA is neither sufficient nor necessary to cause AD*. We speculate that the episodes of hypoxia, followed by reoxygenation causes oxidative stress that injures the hippocampus and adjacent regions. Thus, OSA may render these areas more vulnerable to the (as yet unknown) causative agent in AD, thereby facilitating the pathogenesis of this disease. This is supported by our previous findings of reduced hippocampal volume in the same brains [19].

The present finding that OSA severity significantly predicts Aβ plaque burden aligns with the findings of a PET imaging study that found that OSA resulted in an increase in global Aβ burden and no association with tau-PET [26]. Chronic intermittent hypoxia in rodents mimicking the hypoxia/reoxygenation of OSA increases the deposition of amyloid pathology in the brain [20, 21] and is one possible mechanism for the observed findings. Alternatively, it has been shown in a mouse model that sleep accelerates the clearance of Aβ from the brain [43], with evidence implicating glymphatic clearance during slow wave sleep (for review, see Cordone et al. [44]). Interestingly, the extent of rapid eye movement (REM) sleep and non-rapid eye movement (NREM) slow wave sleep is decreased in both AD [45, 46] and OSA [47], with a recent study finding this relationship in men, but not women with OSA [48]. Mander and colleagues [49] suggested that a self-perpetuating cycle may exist between Aβ deposition and decreased NREM slow wave sleep, where the

deposition of Aβ in the medial prefrontal cortex is the start of the cycle. It may be that the deficiency of NREM sleep in OSA contributes to an inefficient clearance of Aβ from the brain. An interesting extension of this idea is found in individuals with Down syndrome (trisomy 21), who frequently develop a progressive dementia in middle age that is preceded by the deposition of Aβ plaques and NFTs in the cerebral cortex and hippocampal formation [50]. Although these degenerative changes have generally been attributed to the extra copy of chromosome 21 that contains the gene coding for amyloid precursor protein [50], it is notable that the craniofacial structure in Down syndrome causes most individuals to suffer from severe OSA [51]. Since the present study supports the possibility that severe OSA accelerates AD pathogenesis, it is possible that the overexpression of amyloid precursor protein is not the sole factor responsible for the increased Aβ burden in trisomy 21. This possibility could be tested by examining whether the onset of dementia in Down syndrome is affected by the severity of comorbid OSA, and whether the accretion of Aβ in Down syndrome is attenuated by the use of CPAP.

The present study found no effect of CPAP use on the burdens of NFTs or Aβ plaques. This finding is consistent with a PET imaging study that reported that while patients with OSA had a higher global Aβ burden, this burden was not reduced by CPAP use [26]. Nonetheless, the lack of rigorous CPAP compliance data is a significant limitation of both that study and the present study. Treatment variables, including the overall duration of use and nightly usage time could have influenced the association between OSA severity and AD neuropathology. Given that the present CPAP group had a significantly higher BMI and a trend toward higher ODI than non-CPAP users, this result should be interpreted with caution and ought to be explored further in future studies. Especially given the claims that CPAP may provide symptomatic benefit in AD [52, 53].

Brainstem pathology has been observed in people with AD; one study found that all people in the sample had NFTs in the raphe system, and while in the LC, 56% and 62% had NFTs and Aβ plaques, respectively [54]. The higher percentage of Aβ plaques found in that study, compared to the present study, is likely due to the participants in the Parvizi study having clinically diagnosed AD, whereas the present sample had not been diagnosed with any form of dementia. One previous study has investigated the presence of Aβ deposits in the brainstem of nondemented elderly persons and found no Aβ plaques in any subjects [55]. The same study found immunoreactive tau in all 20 brainstems studied; this is supported by the present finding that immunoreactive tau was present in 92% of the pons samples, whereas only 21% had Aβ plaques. As the previous study did not know the OSA status of their samples, it is possible that some of the pathology may have been due to undiagnosed OSA.

While the present study did not find a relationship between OSA severity and brainstem pathology, it is possible that the brainstem nuclei, particularly the LC, are more vulnerable to injury in OSA such that even mild OSA is sufficient to induce pathological tau accumulation. The noradrenergic neurons of the LC are most active during wakefulness, decrease their activation during NREM sleep, and are completely inactive during REM sleep [35]. The LC undergoes substantial neuronal loss in animal models of intermittent hypoxia [56] and intermittent short sleep [57]. Neuronal loss was also seen in the LC of humans with lower oxygen desaturation levels during sleep [38]; however, that study did not investigate the presence of NFTs or Aβ plaques. In OSA, LC neurons may be overactivated by the lack of continuous sleep, leading to mitochondrial oxidative stress and the phosphorylation of tau, making the development of NFTs more likely [58, 59]. Conversely, the neuronal loss in the LC due to short sleep and/or hypoxia may precede the development of tau pathology, resulting in fewer neurons that can contain tau [37]. An alternative explanation for the present finding is that tau pathology in the brainstem increases with age, as has been suggested previously [42]. The present study was unable to test this hypothesis as a control autopsy sample with no OSA (clinically verified by polysomnography) from the same population does not exist.

It should be noted that while the presence of the APOE-ε4 allele is a strong genetic risk factor for AD, the APOE-ε4 allele was not overrepresented in the 26 people with OSA that were successfully tested for APOE status in the present study. Interestingly, a previous meta-analysis found no association between OSA and APOE status [41] suggesting that OSA could accelerate the pathogenesis of AD independently of APOE status. Nonetheless, APOE genotype remains an important factor, and should be included in future analyses of the relationship between OSA and AD.

The present project found a correlation between age and OSA severity, supporting reports that OSA severity is related to increasing age [60], possibly due to degenerative airway changes, including decreased muscle tone and respiratory effort [1], both of which are associated with increasing age [61]. In the present study age significantly predicted the burden of NFTs in the hippocampus of people with OSA, confirming previous reports for elderly populations [42, 62]. Age did not affect the relationship between Aβ burden and OSA severity and a lack of correlation between age and Aβ plaque burden has been reported previously [62].

It is important to acknowledge the limitations of the present study. The project accessed autopsy material that had been archived. Since such tissue is no longer being collected in Iceland, it was not possible to increase the sample size or obtain a control group. A control group would have been difficult to obtain because OSA often goes undiagnosed, and therefore all participants must have undergone a polysomnography to confirm that they did not have OSA. The sample size, while acceptable for a human autopsy study, had limited statistical power, especially for the brainstem analyses which did not have a large enough sample size to control for covariates. Consequently, these results should be considered preliminary, pending confirmation by studies with larger sample sizes. A second limitation is that all tissue in the present study was obtained from Icelandic people; as the genetic diversity in this sample may not be as broad as in the global population, this may limit the generalizability of the findings. ODI was used to measure OSA severity in this study, and while the AHI is the more common measure, the two are highly correlated [63]. The ODI has a stronger emphasis on hypoxemia, which is thought to be a contributor to brain injury, yet as the ODI does not capture hypopneas associated with arousal, the AHI may have been more relevant to the activity of the LC and other brainstem nuclei. The lack of tissue from other brain regions is also a limitation. Typically, AD is diagnosed from an investigation of tissue from the hippocampus and other neocortical regions, including the frontal, parietal, and occipital lobes that develop AD pathology in the later stages of the disease [9,

10]. NFT pathogenesis is considered to follow a six-stage process, with no significant change in pathology in the hippocampus at Stages 5 and 6. These stages constitute increased pathology initially in high-order association areas (Stage 5), and then in secondary and primary association areas (Stage 6). Stages 5 and 6 of NFT burden could not be determined in the present study, as tissue from cortical regions other than the hippocampus was not available. However, Stages 5 and 6 pathology are unlikely to be present without clinical symptoms of dementia, and dementia/AD diagnosis was an exclusion criterion for the present study. Additionally, all of the hippocampal blocks were obtained from the rostroventral hippocampus; thus, we were unable to determine the presence or absence of pathology in other hippocampal regions. A future study could collect tissue from all of the necessary regions in order to overcome this limitation. While none of the people in the study were diagnosed with AD or dementia, it remains possible that some had undiagnosed mild cognitive deficits. Future studies should include neuropsychological tests to control for the possibility of mild cognitive impairment.

In conclusion, the present study has shown that OSA severity is a significant predictor of Aβ plaque burden in the hippocampus after controlling for age, sex, BMI, and CPAP use; although a similar relationship exists for NFT, this can be accounted for by age. OSA is a common disorder and is usually diagnosed decades before AD; interestingly the neuropathological signs of AD also appear decades before the clinical symptoms are detected [42]. We speculate that untreated OSA may account for some of the "cognitively normal" individuals who have been found to have substantial Aβ burdens [64], and are currently considered to be at a prodromal stage of AD.

Supplementary Material

Supplementary material is available at *SLEEP* online.

Acknowledgments

The authors are grateful for the assistance given by The National University Hospital of Iceland and Landspitali Biobank. We are indebted to Professor Emeritus Hannes Blöndal who shared his great knowledge and experience in neuroanatomy when identifying tissue samples.

Funding

J.E.O. was financially supported by the RMIT University Higher Degree by Research Publication Grant.

Conflict of interest statement. None declared.

References

1. Gislason T, *et al*. Obstructive sleep apnoea in adults: epidemiological aspects. In: Annesi-Maesano I, Lundbäck B, Viegi G, eds. *Respiratory Epidemiology*. Sheffield, UK: European Respiratory Society; 2014: 88–105.
2. Senaratna CV, *et al*. Prevalence of obstructive sleep apnea in the general population: a systematic review. *Sleep Med Rev*. 2017;**34**:70–81.
3. Buchanan PR, *et al*. Positive-pressure treatment of obstructive sleep apnea syndrome. *Handb Clin Neurol*. 2011;**98**:421–439.
4. Torelli F, *et al*. Cognitive profile and brain morphological changes in obstructive sleep apnea. *Neuroimage*. 2011;**54**(2):787–793.
5. Canessa N, *et al*. Obstructive sleep apnea: brain structural changes and neurocognitive function before and after treatment. *Am J Respir Crit Care Med*. 2011;**183**(10):1419–1426.
6. Rombouts SA, *et al*. Unbiased whole-brain analysis of gray matter loss in Alzheimer's disease. *Neurosci Lett*. 2000;**285**(3):231–233.
7. Hyman BT, *et al*. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement*. 2012;**8**(1):1–13.
8. McKhann GM, *et al*. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;**7**(3):263–269.
9. Thal DR, *et al*. Sequence of Abeta-protein deposition in the human medial temporal lobe. *J Neuropathol Exp Neurol*. 2000;**59**(8):733–748.
10. Braak H, *et al*. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol*. 2006;**112**(4):389–404.
11. Braak H, *et al*. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. 1991;**82**(4):239–259.
12. Braak H, *et al*. The preclinical phase of the pathological process underlying sporadic Alzheimer's disease. *Brain*. 2015;**138**(Pt 10):2814–2833.
13. Selkoe DJ, *et al*. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med*. 2016;**8**(6):595–608.
14. Chang WP, *et al*. Sleep apnea and the risk of dementia: a population-based 5-year follow-up study in Taiwan. *PLoS One*. 2013;**8**(10):e78655.
15. Ding X, *et al*. Self-reported sleep apnea and dementia risk: findings from the prevention of Alzheimer's disease with vitamin E and selenium trial. *J Am Geriatr Soc*. 2016;**64**(12):2472–2478.
16. Lutsey PL, *et al*. Sleep characteristics and risk of dementia and Alzheimer's disease: the Atherosclerosis Risk in Communities Study. *Alzheimers Dement*. 2018;**14**(2):157–166.
17. Polsek D, *et al*. Obstructive sleep apnoea and Alzheimer's disease: in search of shared pathomechanisms. *Neurosci Biobehav Rev*. 2018;**86**:142–149.
18. Macey PM, *et al*. Brain structural changes in obstructive sleep apnea. *Sleep*. 2008;**31**(7):967–977.
19. Owen JE, *et al*. Neuropathological investigation of cell layer thickness and myelination in the hippocampus of people with obstructive sleep apnea. *Sleep*. 2019;**42**(1). doi:10.1093/sleep/zsy199
20. Ng KM, *et al*. Melatonin reduces hippocampal beta-amyloid generation in rats exposed to chronic intermittent hypoxia. *Brain Res*. 2010;**1354**:163–171.
21. Shiota S, *et al*. Chronic intermittent hypoxia/reoxygenation facilitate amyloid-β generation in mice. *J Alzheimers Dis*. 2013;**37**(2):325–333.
22. Fang H, *et al*. Acute hypoxia promote the phosphorylation of tau via ERK pathway. *Neurosci Lett*. 2010;**474**(3):173–177.
23. Zhang CE, *et al*. Hypoxia-induced tau phosphorylation and memory deficit in rats. *Neurodegener Dis*. 2014;**14**(3):107–116.
24. Liguori C, *et al*. Obstructive sleep apnea is associated with early but possibly modifiable Alzheimer's disease biomarkers changes. *Sleep*. 2017;**40**(5). doi:10.1093/sleep/zsx011
25. Diniz BS, *et al*. Do CSF total tau, phosphorylated tau, and beta-amyloid 42 help to predict progression of mild

cognitive impairment to Alzheimer's disease? A systematic review and meta-analysis of the literature. *World J Biol Psychiatry*. 2008;**9**(3):172–182.
26. Elias A, *et al*. Risk of Alzheimer's disease in obstructive sleep apnea syndrome: amyloid-beta and tau imaging. *J Alzheimers Dis*. 2018;**66**(2):733–741.
27. Yun CH, *et al*. Amyloid burden in obstructive sleep apnea. *J Alzheimers Dis*. 2017;**59**(1):21–29.
28. Carvalho DZ, *et al*. Witnessed apneas are associated with elevated tau-PET levels in cognitively unimpaired elderly. *Neurology*. 2020;**94**(17):e1793–e1802.
29. Leuzy A, *et al*. Tau PET imaging in neurodegenerative tauopathies-still a challenge. *Mol Psychiatry*. 2019;**24**(8):1112–1134.
30. Bubu OM, *et al*. Obstructive sleep apnea and longitudinal Alzheimer's disease biomarker changes. *Sleep*. 2019;**42**(6). doi:10.1093/sleep/zsz048
31. Sharma RA, *et al*. Obstructive sleep apnea severity affects amyloid burden in cognitively normal elderly. a longitudinal study. *Am J Respir Crit Care Med*. 2018;**197**(7):933–943.
32. Jackson ML, *et al*. Severe obstructive sleep apnea is associated with higher brain amyloid burden: a preliminary PET imaging study. *J. Alzheimer's Dis*. 2020; (Preprint):1–7.
33. Giess R, *et al*. Localisation and association of pathomorphological changes at the brainstem in Alzheimer's disease. *Mech Ageing Dev*. 1995;**84**(3):209–226.
34. Ehrenberg AJ, *et al*. Quantifying the accretion of hyperphosphorylated tau in the locus coeruleus and dorsal raphe nucleus: the pathological building blocks of early Alzheimer's disease. *Neuropathol Appl Neurobiol*. 2017;**43**(5):393–408.
35. Murillo-Rodríguez E, *et al*. Basic sleep mechanisms: an integrative review. *Cent Nerv Syst Agents Med Chem*. 2012;**12**(1):38–54.
36. Li Y, *et al*. Effects of chronic sleep fragmentation on wake-active neurons and the hypercapnic arousal response. *Sleep*. 2014;**37**(1):51–64.
37. Zhu Y, *et al*. Selective loss of catecholaminergic wake active neurons in a murine sleep apnea model. *J Neurosci*. 2007;**27**(37):10060–10071.
38. Gelber RP, *et al*. Associations of brain lesions at autopsy with polysomnography features before death. *Neurology*. 2015;**84**(3):296–303.
39. Sanger F, *et al*. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*. 1977;**74**(12):5463–5467.
40. Kontula K, *et al*. Apolipoprotein E polymorphism determined by restriction enzyme analysis of DNA amplified by polymerase chain reaction: convenient alternative to phenotyping by isoelectric focusing. *Clin Chem*. 1990;**36**(12):2087–2092.
41. Varvarigou V, *et al*. A review of genetic association studies of obstructive sleep apnea: field synopsis and meta-analysis. *Sleep*. 2011;**34**(11):1461–1468.
42. Braak H, *et al*. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol*. 2011;**70**(11):960–969.
43. Xie L, *et al*. Sleep drives metabolite clearance from the adult brain. *Science*. 2013;**342**(6156):373–377.
44. Cordone S, *et al*. Sleep and β-amyloid deposition in Alzheimer disease: insights on mechanisms and possible innovative treatments. *Front Pharmacol*. 2019;**10**:695.
45. Vitiello MV, *et al*. Sleep disturbances in patients with Alzheimer's disease: epidemiology, pathophysiology and treatment. *CNS Drugs*. 2001;**15**(10):777–796.
46. Pase MP, *et al*. Sleep architecture and the risk of incident dementia in the community. *Neurology*. 2017;**89**(12):1244–1250.
47. Kim H, *et al*. Effects of long-term treatment on brain volume in patients with obstructive sleep apnea syndrome. *Hum Brain Mapp*. 2016;**37**(1):395–409.
48. Morselli LL, *et al*. Determinants of slow-wave activity in overweight and obese adults: roles of sex, obstructive sleep apnea and testosterone levels. *Front Endocrinol (Lausanne)*. 2018;**9**:377.
49. Mander BA, *et al*. β-amyloid disrupts human NREM slow waves and related hippocampus-dependent memory consolidation. *Nat Neurosci*. 2015;**18**(7):1051–1057.
50. Head E, *et al*. Con: are we ready to translate Alzheimer's disease-modifying therapies to people with down syndrome? *Alzheimers Res Ther*. 2014;**6**(5–8):61.
51. Trois MS, *et al*. Obstructive sleep apnea in adults with Down syndrome. *J Clin Sleep Med*. 2009;**5**(4):317–323.
52. Ancoli-Israel S, *et al*. Cognitive effects of treating obstructive sleep apnea in Alzheimer's disease: a randomized controlled study. *J Am Geriatr Soc*. 2008;**56**(11):2076–2081.
53. Troussière AC, *et al*. Treatment of sleep apnoea syndrome decreases cognitive decline in patients with Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2014;**85**(12):1405–1408.
54. Parvizi J, *et al*. The selective vulnerability of brainstem nuclei to Alzheimer's disease. *Ann Neurol*. 2001;**49**(1):53–66.
55. Fornicola W, *et al*. Alzheimer disease pathology in middle age reveals a spatial-temporal disconnect between amyloid-β and phosphorylated tau. *Open Neurol J*. 2014;**8**:22–26.
56. Zhang JH, *et al*. Apnea produces neuronal degeneration in the pons and medulla of guinea pigs. *Neurobiol Dis*. 2010;**40**(1):251–264.
57. Zhu Y, *et al*. Intermittent short sleep results in lasting sleep wake disturbances and degeneration of locus coeruleus and orexinergic neurons. *Sleep*. 2016;**39**(8):1601–1611.
58. Iijima-Ando K, *et al*. Loss of axonal mitochondria promotes tau-mediated neurodegeneration and Alzheimer's disease-related tau phosphorylation via PAR-1. *PLoS Genet*. 2012;**8**(8):e1002918.
59. Zhang J, *et al*. Extended wakefulness: compromised metabolics in and degeneration of locus ceruleus neurons. *J Neurosci*. 2014;**34**(12):4418–4431.
60. Butner KL, *et al*. Association of obstructive sleep apnea severity with exercise capacity and health-related quality of life. *N Am J Med Sci*. 2013;**5**(6):362–366.
61. Eikermann M, *et al*. The influence of aging on pharyngeal collapsibility during sleep. *Chest*. 2007;**131**(6):1702–1709.
62. Price JL, *et al*. Neuropathology of nondemented aging: presumptive evidence for preclinical Alzheimer disease. *Neurobiol Aging*. 2009;**30**(7):1026–1036.
63. Ernst G, *et al*. Difference between apnea-hypopnea index (AHI) and oxygen desaturation index (ODI): proportional increase associated with degree of obesity. *Sleep Breath*. 2016;**20**(4):1–9.
64. Price JL, *et al*. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann Neurol*. 1999;**45**(3):358–368.