

## **Orexin-A is Associated with Increases in Cerebrospinal Fluid Phosphorylated-Tau in Cognitively Normal Elderly Subjects**

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## **ABSTRACT:**

### **STUDY OBJECTIVES:**

To evaluate the role of orexin-A with respect to cerebrospinal fluid (CSF) Alzheimer disease (AD) biomarkers, and explore its relationship to cognition and sleep characteristics in a group of cognitively normal elderly individuals.

### **METHODS:**

Subjects were recruited from multiple community sources for National Institutes of Health supported studies on normal aging, sleep and CSF biomarkers. Sixty-three participants underwent home monitoring for sleep-disordered breathing, clinical, sleep and cognitive evaluations, as well as a lumbar puncture to obtain CSF. Individuals with medical history or with magnetic resonance imaging evidence of disorders that may affect brain structure or function were excluded. Correlation and linear regression analyses were used to assess the relationship between orexin-A and CSF AD-biomarkers controlling for potential sociodemographic and sleep confounders.

### **RESULTS:**

Levels of orexin-A, amyloid beta 42 (A $\beta$ 42), phosphorylated-tau (P-Tau), total-tau (T-Tau), Apolipoprotein E4 status, age, years of education, reported total sleep time, number of awakenings, apnea-hypopnea indices (AHI), excessive daytime sleepiness, and a cognitive battery were analyzed. Subjects were  $69.59 \pm 8.55$  years of age, 57.1% were female, and 30.2% were apolipoprotein E4+. Orexin-A was positively correlated with A $\beta$ 42, P-Tau, and T-Tau. The associations between orexin-A and the AD-biomarkers were driven mainly by the relationship between orexin-A and P-Tau and were not influenced by other clinical or sleep characteristics that were available.

### **CONCLUSIONS:**

Orexin-A is associated with increased P-Tau in normal elderly individuals. Increases in orexin-A and P-Tau might be a consequence of the reduction in the proportion of the deeper, more restorative slow wave sleep and rapid eye movement sleep reported with aging.

### **CLINICAL TRIAL REGISTRATION:**

Clinicaltrials.gov registration number NCT01962779.

### **Key words: 8**

Alzheimer's disease, Sleep, Orexin-A, Cerebrospinal Fluid, Sleep Disordered Breathing, Amyloid Beta, Tau, Aging

### **INTRODUCTION:**

The '*Amyloid Cascade Hypothesis*'<sup>1</sup> posits that the accumulation and deposition of amyloid beta (A $\beta$ ) in the brain is the initiating pathological event in Alzheimer's disease (AD). In humans, a growing body of evidence suggests that synaptic activity is associated with increased production of A $\beta$ <sup>2-8</sup>. During sleep, the brain maintains its connectivity during light sleep<sup>9-11</sup> but reduces its metabolic and electric activity with increasing depth of non REM (NREM) sleep<sup>9-13</sup>, suggesting that brain soluble A $\beta$  levels may fluctuate with a diurnal

pattern consistent with higher neuronal activity during wakefulness and decreased neuronal activity during NREM sleep<sup>14</sup>. Evidence from this A $\beta$  diurnal pattern has been reported in some human studies but not others<sup>15-19</sup>.

Orexin-A (hypocretin 1), a neuropeptide produced by lateral hypothalamic neurons<sup>20</sup>, is involved in the regulation of the sleep-wake cycle by increasing arousal levels<sup>21-23</sup> and has been suggested to promote A $\beta$  production and amyloid deposition in transgenic mice<sup>16</sup>. The relationship between the orexinergic system and the AD neurodegenerative process in humans has been analyzed by a variety of cross-sectional studies in different clinical populations showing so far inconclusive results<sup>24-29</sup>. Whether increases in A $\beta$  production are directly related to orexin-A or are secondary to changes in the sleep-wake cycle is also unknown.

Based on this preliminary evidence, the aims of this study were: (1) to investigate the involvement of the orexinergic system in A $\beta$ 42 dynamics by measuring cerebrospinal fluid (CSF) orexin-A and AD biomarker levels in a large group of non-demented elderly, first globally and then by defining a group of *controls* and *increased risk* for AD by their core CSF biomarker profile; (2) evaluate the relationships between the orexinergic system and cognition; and, (3) elucidate the role of the orexinergic system, A $\beta$ 42 and tau with respect to other characteristics of sleep such as total sleep time (TST), number of awakenings, excessive daytime sleepiness or sleep disordered breathing (SDB).

## **METHODS**

### **Subject recruitment**

67 non-demented elderly were recruited at NYU Center for Brain Health from active National Institutes of Health (NIH) supported longitudinal studies of normal aging, Mild Cognitive Impairment (MCI) and CSF diagnostic AD-biomarkers that have been ongoing between 1998 and 2015. All subjects agreed to undergo additional home monitoring for SDB, a detailed sleep interview and filled out the Epworth Sleepiness Scale (ESS)<sup>38</sup>. Subjects had previously been recruited from multiple community sources including random sampling using voter registration records. Individuals with medical conditions or history of significant conditions that may affect the brain structure or function, such as stroke, uncontrolled diabetes, traumatic brain injury, any neurodegenerative diseases, active major depression, as well as MRI evidence of intracranial mass or infarcts were excluded from the parent studies. Sleep complaints were not part of the inclusion or exclusion criteria of any of the NIH studies that the subjects were recruited from, nor were subjects referred to the study from the NYU Sleep Disorders Clinic that performed the later sleep analyses.

### **Clinical and diagnostic evaluation**

Subjects received a standardized diagnostic assessment that included medical, psychiatric, and neurologic evaluations. The selected subjects were not on active treatment for SDB with CPAP or dental appliances. Eligibility requirements for the present study included having had CSF collected from lumbar puncture (LP) and a diagnostic structural MRI scan completed prior to the sleep examination (average time interval between the sleep study and the lumbar puncture 0.9 $\pm$ 1.1 years). Presence of the ApoE4 genotype was determined using standard polymerase chain reaction procedures.

### **Cognitive evaluation**

All subjects were administered a standard neuropsychological test battery, which has published norm values<sup>30</sup>. The measures included subtests of the Guild Memory Scale: verbal paired associates (initial: PRDI, delayed: PRDD, and immediate: PARI), delayed paragraph recall subtest (PARD) and the Wechsler Memory Scale Revised<sup>31</sup>: Logical Memory subtests (Logic I and II), to measure declarative memory. Subtests of the

Wechsler Intelligence Scale Revised to assess working memory (digits forward: WAISDIG-F and backward: WAISDIG-B)<sup>32</sup>. The Digit Symbol Substitution Test (DSST)<sup>32</sup> to evaluate psychomotor speed. Trails A Test to evaluate attention and Trails B Test to evaluate executive function<sup>33</sup>. Category fluency (animals and vegetables) and the Boston Naming Test (BNT)<sup>34</sup> were used to evaluate language. We subsequently grouped cognitive tests into episodic memory: (PARI, PRDI, Logic I, PARD, PRDD, Logic II, DESN) and executive functions (WAISDIGB, DSST, category animals and vegetables, TMT B) domains. The score for each domain was an average of z-scores of all tests comprised. The average of both domains used was created as a measure of global overall cognition. The Mini Mental State Examination<sup>35</sup> as an additional global measure of cognition was also included.

### **Cerebrospinal fluid**

Lumbar punctures were performed between 11:00 AM and 01:00 PM using a 25-gauge needle guided by fluoroscopy. All CSF samples were kept on ice until centrifuged for 10 minutes at 1500g, at 4°C. Samples were aliquoted to 0.25 mL polypropylene tubes and stored at -80 °C until assayed. CSF was analyzed for A $\beta$ 38, A $\beta$ 40 and A $\beta$ 42 using the Meso Scale Discovery (MSD) A $\beta$  Triplex assay as described by the manufacturer (MSD, Gaithersburg, Md., USA). CSF phosphorylated tau at threonine 181 (P-tau) and total tau (T-tau) were blindly analyzed in batch mode using enzyme-linked immunosorbent assays<sup>36;37</sup>. Orexin-A was measured using an in-house RIA with minor modifications<sup>29</sup>. The CSF *increased risk* for AD group was defined as a CSF P-tau/A $\beta$ 42 ratio  $\geq 0.11$ , while the *control* group was defined as a CSF P-tau/A $\beta$ 42 ratio  $< 0.11$ . This value was based on a separate NYU data set of 171 cognitively normal subjects and 28 AD patients modeled to determine the optimal cut-off for diagnostic prediction of AD (data not published).

### **Sleep Evaluation**

A full sleep evaluation was performed on all subjects, which included a sleep interview, detailed snoring history, and self-administration of the ESS<sup>38</sup>. Home monitoring of SDB was completed using either an ARES Unicorder<sup>39</sup> or an Embletta MPR<sup>40</sup> systems during a 2-night period. The variables collected included were: 1) the apnea/hypopnea index with 4% desaturation (AHI4%), defined as the sum of all apneas ( $>90\%$  reduction in airflow for  $>10$  seconds) and all hypopneas ( $>30\%$  reduction in airflow) associated with  $>4\%$  O<sub>2</sub> desaturation divided by the total time where both flow and oximetry signals were valid; 2) the AHIall, which was defined as the sum of all apneas and all hypopneas identified divided by the total time where there was a valid flow signal irrespective of O<sub>2</sub> saturation; and, 3) mean SpO<sub>2</sub> saturation during the night. Both systems and AHI indices have been compared with the recommended definitions of AHI based on full in laboratory polysomnography that included electroencephalogram measures of sleep and show good comparability<sup>39;40</sup>. Reported total sleep time (TST) duration was assessed using one question: 'During the past month, how many hours of sleep did you usually get each night, what is your best estimate'? Sleep fragmentation was assessed using a second question. 'During the past month, how many times do you wake up each night, on average'?

### **Statistical analysis**

Regression-based z-scores corrected for age, sex, race and education, derived from our normative sample<sup>30</sup> were used for comparisons of cognitive measures with CSF orexin-A values. The Bonferroni correction was used to adjust for multiple comparisons. Non-parametric correlation analyses were used to assess for the relations between continuous dependent (CSF AD-biomarkers) and explanatory variables (orexin-A), as well as to obtain correlation coefficients. Multiple linear regression were used to create a *sleep prediction model* for A $\beta$ 42, P-Tau and T-tau, the AD-biomarker being the dependent variable and orexin-A, AHI4%, AHIall, ESS, BMI, age, TST and number of awakenings as independent sleep variables. The linear regression model chosen was the one with the highest coefficient of determination (R<sup>2</sup>) in which all variables maintained a significant association ( $p < 0.05$ ) or a trend ( $p < 0.1$ ). Analyses were done with SPSS 20.0 (Chicago, IL, USA).

## RESULTS:

### Participant characteristics:

Demographic characteristics of all subjects (n=67) are shown in Table 1. Subjects were aged 69.8±8.9 years, 56.7% were female, 30% were ApoE4+. When comparing CSF defined *controls* (n=49) with *increased risk* for AD subjects (n=18), statistically significant differences between groups were observed in BMI and presence of ApoE4 status.

	All subjects (n=67)	Controls (n=49)	Increased risk for AD (n=18)
Age (y) (mean ± SD)	69.83±8.85	70.74±8.74	67.35±8.94
Female (%)	56.7%	53.1%	66.7%
BMI (mean ± SD)	26.64±4.59	25.80±3.98	<b>28.93±5.44*</b>
Years of education (mean ± SD)	16.49±2.33	16.39±2.42	16.78±2.10
Hypertension (%)	46.3%	46.9%	44.4%
Cardiovascular disease (%)	7.5%	8.2%	5.6%
Diabetes (%)	6.0%	6.1%	5.6%
Thyroid disease (%)	23.9%	26.5%	16.7%
Geriatric Depression Score (mean ± SD)	1.99±0.36	1.98±0.38	2.00±0.34
ApoE4+ (%)	29.9%	20.4%	<b>55.6%**</b>

**Table 1.** Abbreviations: BMI, Body Mass Index; ApoE4+, Apolipoprotein E4 positive. Results are reported in mean ± standard deviation.

### CSF AD biomarker levels and orexin-A

Summary of CSF levels of A $\beta$ 38/40/42, P-tau, T-tau and orexin-A are shown in Table 2. When comparing CSF defined *controls* with *increased risk* for AD subjects using BMI and ApoE4 status as covariates, only CSF levels of A $\beta$ 42 differed significantly between groups (F=15.7, p<0.01). No other statistically significant differences were found between *controls* and *increased risk* for AD subjects in any other biomarkers of risk for AD or in orexin-A levels.

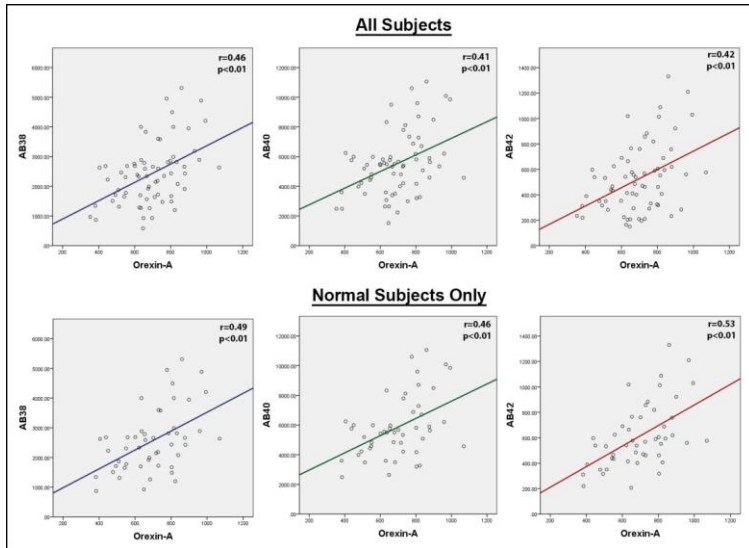
In all subjects, including the *controls* and the *increased risk* for AD groups, significant correlations were found between all CSF biomarkers and orexin-A levels (A $\beta$ 38: r=0.43, p<0.01; A $\beta$ 40: r=0.37, p<0.01; A $\beta$ 42: r=0.39, p<0.01; P-Tau: r=0.52, p<0.01; T-Tau: r=0.37, p<0.01). Excluding the *increased risk* for AD subjects strengthened all correlations (A $\beta$ 38: r=0.49, p<0.01; A $\beta$ 40: r=0.46, p<0.01; A $\beta$ 42: r=0.53, p<0.01; P-Tau: r=0.54, p<0.01; T-Tau: r=0.41, p<0.01). In the *increased risk* for AD group, there were no significant associations between CSF biomarkers and orexin-A, although there was a trend for T-Tau (r=0.46, p=0.052).

In the multiple linear regression models analyses, the best fit sleep model for prediction of A $\beta$ 42 in all subjects (*controls* and *increased-risk*) included: orexin-A ( $\beta$ =.34, p<0.01) age ( $\beta$ =.24, p<0.05), BMI ( $\beta$ =-.31, p<0.01), and AHI4% ( $\beta$ =.24, p<0.05) (F=8.0, R<sup>2</sup>=.30, p<0.01). For T-tau, the best fit sleep model included age ( $\beta$ =.27, p<0.05), number of awakenings ( $\beta$ =.18, p<0.1) and orexin-A ( $\beta$ =.41, p<.01) (F=7.36, R<sup>2</sup>=.26, p<0.01). For P-Tau no other sleep variables significantly contributed to the model aside from orexin-A. For the control group subjects only, the best fit sleep model for prediction of A $\beta$ 42 included orexin-A ( $\beta$ =.64, p<0.01) age ( $\beta$ =.26, p<.05), AHIall ( $\beta$ =.28, p<0.05) (F=11.13, R<sup>2</sup>=.43, p<.01). For P-Tau, the best fit model included orexin ( $\beta$ =0.58, p<0.01) and AHIall ( $\beta$ =.22, p<0.1) (F=12.2, R<sup>2</sup>=.35, p<0.01). For T-tau, the best model included orexin-A ( $\beta$ =.51, p<.01) and age ( $\beta$ =.26, p<.05) (F=8.7, R<sup>2</sup>=.28, p<0.01).

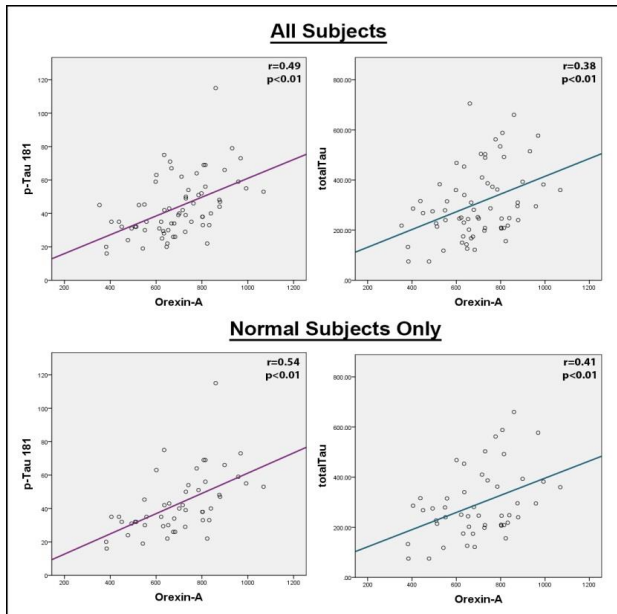
CSF Biomarker	All subjects (n=67)	controls (n=49)	increased risk for AD (n=18)
A $\beta$ 40	5510.43 ± 2132.90	5845.48 ± 2096.32	4598.36 ± 2012.27
A $\beta$ 42	521.80 ± 267.24	612.46 ± 252.08	274.99 ± 98.23**

P-Tau	43.67 ± 17.85	42.75 ± 18.41	46.17 ± 16.46
T-Tau	305.73 ± 142.68	292.01 ± 137.15	343.09 ± 154.63
Orexin-A	692.18 ± 156.82	696.39 ± 167.00	680.72 ± 128.62

**Table 2.** Abbreviations: CSF, Cerebrospinal Fluid; A $\beta$ , amyloid beta; P-Tau, Phosphorylated Tau; T-Tau, Total Tau. Results are reported in mean  $\pm$  standard error.



**Figure 1.** Scatter plots of CSF Orexin and A $\beta$ 38, A $\beta$ 40, and A $\beta$ 42 with all subjects and with normal subjects only.



**Figure 2.** Scatter plots of CSF Orexin and P-Tau and T-Tau with all subjects and with normal subjects only.

### **Cognition and orexin-A**

Cognitive characteristics of all subjects are shown in Table 3. All participants had at least 12 years of education (mean value: 16.49 $\pm$ 2.33) and a MMSE score >26 (mean value: 29.3 $\pm$ 1.0). 4 participants had 2 or more cognitive test scores 2 standard deviations (SD) below the mean for their age, race, and education-matched peers. 4 participants were diagnosed as MCI with a Clinical Dementia Rating (CDR) scale of 0.5 by the study clinician. When comparing the CSF defined *controls* with the *increased risk* for AD subjects using

BMI and ApoE4 status as covariates, there were no significant differences in cognitive variables between groups. When comparing all subjects, *controls* and *increased risk* for AD groups cognitive scores with orexin-A levels, the only significant positive correlation found was between orexin-A and Logic II ( $r=0.28$ ,  $p=0.024$ ). Excluding *increased risk* for AD subjects strengthened this correlation ( $r=0.38$ ,  $p=0.007$ ). Additionally, after the *increased-risk* subjects were excluded, there was a significant positive correlation between orexin-A and BNT ( $r=0.28$ ,  $p=0.048$ ), and a trend towards lower z-scores on WAISDIG-F ( $r=-0.29$ ,  $p=0.052$ ). Orexin-A levels were not significantly correlated to any other cognitive variables, nor were these associations positive after adjusting for multiple comparisons in the above analyses. Moreover, orexin-A levels were not significantly correlated to any measure of global cognition (episodic memory, executive function and global cognitive function).

	<b>All subjects</b>	<b>Controls</b>	<b>increased risk for AD</b>
MMSE (raw)	29.22 ± 0.14	29.30 ± 0.15	29.15 ± 0.25
PRDI (z-scores)	0.47 ± 0.19	0.39 ± 0.19	0.56 ± 0.35
PRDD (z-scores)	0.63 ± 0.19	0.46 ± 0.20	0.80 ± 0.36
PARI (z-scores)	0.086 ± 0.14	-0.12 ± 0.15	0.30 ± 0.25
PARD (z-scores)	0.16 ± 0.14	-0.13 ± 0.14	0.46 ± 0.25
Logic2 (z-scores)	-0.031 ± 0.17	-0.27 ± 0.17	0.21 ± 0.31
WAISDIG-F (z-scores)	-0.34 ± 0.18	-0.088 ± 0.19	-0.60 ± 0.33
WAISDIG-B (z-scores)	-0.31 ± 0.15	-0.23 ± 0.16	-0.39 ± 0.28
DSST (z-scores)	0.31 ± 0.14	0.30 ± 0.14	0.32 ± 0.25
Trails A Test (z-scores)	-0.31 ± 0.13	0.087 ± 0.14	-0.70 ± 0.25
Trails B Test (z-scores)	-0.22 ± 0.12	-0.027 ± 0.12	-0.41 ± 0.22
Category fluency animals (z-scores)	0.37 ± 0.14	-0.028 ± 0.14	0.76 ± 0.25
Category fluency vegetables (z-scores)	0.19 ± 0.15	-0.12 ± 0.15	0.51 ± 0.27
BNT (z-scores)	-0.14 ± 0.14	-0.24 ± 0.14	-0.042 ± 0.26
Global Episodic Memory (z-scores)	0.30 ± 0.11	0.11 ± 0.11	0.49 ± 0.20
Global Executive Function (z-scores)	0.068 ± 0.071	-0.036 ± 0.074	0.17 ± 0.13
Global Cognition (z-scores)	0.16 ± 0.078	0.018 ± 0.079	0.31 ± 0.14

**Table 3.** Abbreviations: MMSE, Mini Mental State Examination; PRDI, verbal paired associates initial; PRDD, verbal paired associates delayed; PARI, verbal paired associates immediate; PARD, delayed paragraph recall subtest; Logic2, logical memory subtest; WAISDIG-F, digits forward; WAISDIG-B, digits backwards; DSST, Digit Symbol Substitution Test; BNT, Boston Naming Test. Results are reported in mean ± standard error

### **TST, SDB, ESS and orexin-A**

Summary of objective (AHI4%, AHIall and mean SpO<sub>2</sub>) and subjective (reported TST, reported number of awakening and ESS) sleep indices are shown in Table 4. Among the 67 participants, 22 subjects were considered free of SDB (AHI4% < 5), 30 had mild SDB (AHI4% 5-14.99), and 15 had moderate to severe SDB (AHI4% > 15). 8 subjects were considered short sleepers (< 6 hours), 41 were normal sleepers (6-8 hours) and 17 were long sleepers (> 8 hours). Only 8 subjects complained of excessive sleepiness (ESS > 10). Of all subjects, 4 were being treated with fluoxetine, 1 was taking bupropion, 2 were taking lorazepam, 1 was taking trazodone, 2 were taking diazepam and 2 were taking non-prescription sleep medication (melatonin). When comparing CSF defined controls with *increased risk* for AD subjects using BMI and ApoE4 status as covariates, there were no significant differences in the sleep variables between groups, although there was an higher number of awakenings in the *increased-risk* for AD group ( $F=4.1$ ,  $p<0.05$ ). In all subjects, as well as in the control or the *increased risk* for AD groups, there were no significant correlations found between orexin-A levels and any of the sleep variables.

<b>Sleep characteristic</b>	<b>All subjects</b>	<b>Normal</b>	<b>At risk for AD</b>
TST (mean ± SD)	7.10±1.10	7.14±1.11	6.89±1.16
Sleep Awakenings (mean ± SE)	1.76±1.38	1.48±1.15	2.31±1.72*
ESS (mean ± SE)	5.55±3.36	5.67±3.30	5.22±3.59
AHI4% (mean ± SE)	11.30±12.30	11.26±12.15	11.49±12.85
AHIall (mean ± SE)	24.20±14.60	23.78±14.73	25.02±14.60
Mean nocturnal SpO <sub>2</sub> (mean ± SE)	94.00%±6.20	94.66%±1.74	94.78%±1.31

**Table 4.** Abbreviations: TST: reported total sleep time; ESS: Epworth sleepiness scale; AHI4%: apnea/hypopnea index with 4% oxygen desaturation; AHIall: sum of apneas and hypopneas occurring independent of oxygen desaturation Results are reported in mean  $\pm$  standard error/deviation

## **DISCUSSION**

This study is unique in systematically testing multiple CSF AD-biomarkers and comparing them to orexin-A levels in a group of non-demented elderly with available objective and subjective measurements of sleep. CSF orexin-A levels were positively correlated with A $\beta$ 38/40/42, P-Tau and T-tau and the associations were strengthened when subjects with a CSF biomarker profile indicative of an increased risk for AD were excluded, which suggests that these associations may decrease in the presence of amyloid deposition, axonal degeneration or tau pathology. Including measures of intermittent hypoxia, sleep fragmentation and sleep quality such as AHI4%, AHIall or number of awakening increased the AD biomarker prediction models which would indicate a more complex modulation of A $\beta$ 42 and tau pathology by sleep than previously thought. Less than 10% of subjects in this sample were receiving psychotropic medication which may have had influence on orexin-A levels or neuronal activity in previous studies.

Previous publications are conflicting with reports of positive or no associations between orexin-A and other CSF biomarkers of risk for AD (Table 5). The first study that related CSF A $\beta$ 42 to orexin-A was performed in a group of six AD patients (mean age 71, range 64-77) and six healthy volunteers (mean age 71, range 59-85)<sup>25</sup>. CSF biomarkers were determined at individual time points with a lumbar catheter during 8 hours. Orexin-A showed a circadian rhythm with amplitude of 11.5 pg/ml that was positively associated with A $\beta$ 42 when the whole sample (AD and controls) was analyzed. No other analytes were tested. A second study, performed in a group of 12 Down syndrome (DS) patients (mean age 41 $\pm$ 11) and 20 age-matched controls (mean age 41 $\pm$ 15)<sup>26</sup>, also found a positive correlation between orexin-A and A $\beta$ 38/40 in both controls and DS patients, while A $\beta$ 42 only correlated with orexin-A in the control group. A correlation of orexin-A with T-tau was also reported in the DS group but not in the controls. The third study was performed in a sample of 37 AD patients (mean age 72.30, range 49.80–89.49), 16 MCI-AD-decliners (mean age 73.20, range 57.39-84.39), 38 other dementias (mean age 66.84, range 32.07-85.15) and 15 elderly non-demented controls with narcolepsy-cataplexy (mean age 65.59, 54.04-86.38)<sup>28</sup>. A positive correlation between orexin-A and A $\beta$ 42 was found only in the AD group. In contrast, three studies have found associations between orexin-A and tau but not with A $\beta$ 42. The first study analyzed a group of 48 AD patients and 28 non-demented inpatient controls admitted for suspected subarachnoid hemorrhage or chronic polyneuropathy<sup>24</sup>. CSF orexin-A levels were directly correlated with T-tau only in the AD group. The second study included two different groups of AD patients (n=10 and 17, mean age 66.4 $\pm$ 7.2 and 78.8 $\pm$ 7.9 respectively) and compared them with two groups of depressed patients in full remission (n=10 and 8, mean age 66.3 $\pm$ 9.3 and 70.5 $\pm$ 6.5 respectively)<sup>41</sup>. When the whole group was analyzed, orexin-A was related with increases in P-Tau/T-Tau and to a lesser extent to A $\beta$ 40 but not to A $\beta$ 42. The third study analyzed a group of 26 AD patients, 18 patients with Lewy Body dementia and 24 non-demented controls<sup>27</sup>. Orexin-A was linked to T-tau in female non-demented controls whereas associations between orexin-A and A $\beta$ 42 were absent in all groups regardless of gender. Finally, one study performed in a group of 33 AD patients and 33 healthy volunteers found no significant associations between A $\beta$ 42 or tau<sup>29</sup>. The results from the different studies are hard to interpret due to the small sample sizes, heterogeneity of comparison groups, presence or absence of psychotropic medication, lack of objective sleep assessments, wide age ranges and the possibility that the orexinergic system is affected by the neurodegenerative process itself, as shown by a postmortem study that reported a 40% decreased cell number and 14% lower CSF orexin levels in advanced AD patients<sup>42</sup>, which may explain some of the negative findings. Altogether, these results suggest that the relationship between orexin-A and AD biomarkers may vary depending on age, clinical diagnoses, sleep quality, medication, dementia severity, hypothalamic damage and presence or absence of amyloid plaques.



Author	Subjects	n	Age	Orexin -A	A $\beta$ 42	A $\beta$ 40	A $\beta$ 38	P-tau	T-tau
Slats, D (2012)	AD	6	71	407	Not specific d †	n.a	n.a	n.a	n.a
	Outpatient controls	6	71	401					
	Assay				RIA <sup>§</sup>	Innobia §§	ELISA <sup>§§§</sup>	n.a	n.a
Portelius, E (2014)	DS patients	12	41± 11	488	n.s	r=0.78 ††	r=0.85 †††	n.s.	r=0.72 ††
	Inpatient controls*	20	40± 15	716	r=0.56 ††	r=0.58 ††	r=0.78 †††	n.s.	n.s.
	Assay				RIA <sup>§</sup>	MSD <sup>§§§§</sup>	MSD <sup>§§§§</sup>	MSD <sup>§§§</sup> §	Innotest ELISA <sup>§§</sup>
Liguori, (2014) C23	Mild AD	21	71.7± 6.3	137.69	n.s	n.a	n.a.	n.s.	n.s.
	Moderate AD	27	69.5±8.4	154.36	n.s	n.a	n.a.	r=0.56 <sup>†</sup>	r=0.64 ††
	Inpatient controls	15	70.4± 9.9	131.03	n.s	n.a	n.a.	Not reported	n.s
	Assay				EIA <sup>§</sup>	n.a	Innotest ELISA <sup>§§</sup>	n.a	Innotest ELISA <sup>§§</sup>
Deuschle, M ** (2014) (Detection sample)39	AD patients	17	78.7±7.7	417.0	n.s	r=0.45 ‡	n.a.	r= 0.57 †	r=0.53 †
	Outpatient Controls***	8	70.5±6.5	408.0	n.s	r=0.76 †	n.a.	r= 0.88 ††	r=0.90 ††
	Assay				RIA <sup>§</sup>	Innotest ELISA <sup>§§</sup>	Innotest ELISA <sup>§§</sup>	n.a	n.a
Deuschle, M** (2014) (Replication sample)39	AD patients	10	66.4±7.2	248.0	n.s	r=0.64 †	n.a.	r= 0.72 †	r=0.59 ‡
	Outpatient Controls***	10	66.3±9.3	238.0	n.s	n.s	n.a.	r= 0.55 ‡	n.s
	Assay				RIA <sup>§</sup>	Innotest ELISA <sup>§§</sup>	Innotest ELISA <sup>§§</sup>	n.a	n.a
Wennstrom M 26 (2012)	AD patients	26	73±6		n.s.	n.a	n.a.	n.s.	n.s.
	DLB patients	18	74±7		n.s.			r=0.753 (M only) †	n.s.
	Outpatient controls***	24	72±8		n.s.			r=0.701 (F only) ††	r=0.683 (F only) ††
	Assay				RIA <sup>§</sup>	n.a	n.a	n.a	n.a
Dauvilliers YA 27 (2014)	AD patients	37	72.3	451.0	r=0.45, ††	n.a.	n.a.	n.s.	n.s.
	MCI due to AD	16	73.2	503.75	n.s.			n.s.	n.s.
	Other dementia	38	66.8	386.0	n.s.			n.s.	n.s.
	NC with no cognitive abnormalities	15	65.6	34.0	n.s.			n.s.	n.s.
	Assay				RIA <sup>§</sup>	Innotest ELISA <sup>§§</sup>	n.a	n.a	Innotest ELISA <sup>§§</sup>

Schmidt, FM 2013	AD patients	33	73.8±8.1	86.5	n.s.			n.s.	n.s.
	Inpatient controls- no psych/neuro problems	33	52.0± 17.2	87.9	n.s.			n.s.	n.s.
	Assay			RIA <sup>§</sup>	Innotest ELISA <sup>§§</sup>	n.a	n.a	Innotest ELISA <sup>§§</sup>	Innotest ELISA <sup>§§</sup>

**Table 5.** Abbreviations are as follows. AD: Alzheimer’s Disease; DS= Down’s syndrome; n.s.: not statistically significant; n: number of subjects; SD: standard deviation; n.a.: not applicable. Age reported as mean age±standard deviation; MCI: mild cognitive impairment; NC: narcolepsy-cataplexy. \*Control subjects recruited from the Surgical Stress Study, psychiatrically healthy inpatients coming for knee arthroplastic surgery. \*\*Procedures performed in an initial “detection” sample and then replicated in the “replication” sample, both samples were distinct from one another \*\*\*MDD patients drug free except lorazepam, depressed patients were in full remission. †p<0.05 ††p<0.01 †††p<0.001 †0.1>p>0.05 §Radioimmunoassay/Enzyme-linked Immunosorbent Assay, Phoenix Pharmaceuticals, Mountain View, CA, USA §§Enzyme-linked Immunosorbent Assay, Innogenetics, Ghent, Belgium §§§The Genetics Company, Schlieren, Switzerland §§§§Meso Scale Discovery A $\beta$  Triplex Assay, Gaithersburg, MD, USA

The clinical relevance of the positive associations we observed between A $\beta$  or tau species with orexin-A in non-demented elderly is unknown. Possible explanations for these results include that orexin-A acts directly on synaptic activity and remodeling increasing the production of A $\beta$  and tau species prior to neurodegeneration, or, that orexin-A may increase AD biomarkers as a result of modulating the sleep-wake cycle by either decreasing the total amount of NREM sleep<sup>43</sup> or increasing inappropriate transitions between wakefulness, NREM and REM<sup>44</sup>. In a study performed in APP/PS1/OR<sup>-/-</sup> amyloid transgenic mice, in which the orexin gene was knocked out<sup>45</sup>, loss of orexin resulted in decreased wakefulness and a subsequent reduction in amyloid pathology. Focal overexpression of orexin did not alter the amount of A $\beta$  pathology in these mice, while sleep deprivation increased the amount of A $\beta$  plaques. These findings support the hypothesis that it is the effects of orexin-A on the sleep-wake cycle and not the expression of the neuropeptide itself that modulates A $\beta$  pathology. In humans, one postmortem study has verified the coexistence of narcolepsy and AD, which demonstrates that AD pathology can also develop in the absence of orexin<sup>46</sup> and would support this interpretation. Our results, indicating that other sleep characteristics improve the CSF prediction models are also in line with these hypotheses.

Limitations of this study include the cross-sectional nature of the findings, the use of a CSF P-tau/A $\beta$ 42 ratio that is laboratory dependent and not generally applicable to other cohorts, and the time interval between the sleep studies and the LP. However, for most key findings, consistent associations were found in the whole sample including *controls* and at *increased-risk* groups supporting the main conclusions. Additionally, controlling for the time between both procedures did not modify the associations. The results of this study should be validated in independent cohorts with in-lab measures of sleep architecture and LPs performed immediately after the sleep studies.

In summary, we found evidence that CSF orexin-A levels are positively associated with A $\beta$ , P-tau and T-Tau in a group of non-demented elderly. The strength of the correlations increased when subjects at a theoretical increased risk for AD were excluded from the model which indicates that these relationships may be more commonly found before the onset of significant plaque pathology or axonal degeneration occur. Including SDB indices, and number of awakenings improved the prediction models which suggest a more complex role of sleep in AD.

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#### Reference List

- (1) Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 1992;256:184-185.
- (2) Buckner RL, Snyder AZ, Shannon BJ et al. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *Journal of Neuroscience* 2005;25:7709-7717.
- (3) Gusnard DA, Raichle ME, Raichle ME. Searching for a baseline: functional imaging and the resting human brain. *Nat Rev Neurosci* 2001;2:685-694.
- (4) Bero AW, Yan P, Roh JH et al. Neuronal activity regulates the regional vulnerability to amyloid- $\beta$  deposition. *Nature Neuroscience* 2011;14:750-756.
- (5) Cirrito JR, Yamada KA, Finn MB et al. Synaptic activity regulates interstitial fluid amyloid- $\beta$  levels in vivo. *Neuron* 2005;48:913-922.
- (6) Jagust WJ, Mormino EC. Lifespan brain activity,  $\beta$ -amyloid, and Alzheimer's disease. *Trends in Cognitive Sciences* 2011;15:520-526.
- (7) Gouras GK, Relkin NR, Sweeney D, Munoz DG, Mackenzie IR, Gandy S. Increased apolipoprotein E epsilon 4 in epilepsy with senile plaques. *Ann Neurol* 1997;41:402-404.
- (8) Mackenzie IR, Miller LA. Senile plaques in temporal lobe epilepsy. *Acta Neuropathol* 1994;87:504-510.
- (9) Horovitz SG, Braun AR, Carr WS et al. Decoupling of the brain's default mode network during deep sleep. *Proc Natl Acad Sci U S A* 2009;106:11376-11381.
- (10) Larson-Prior LJ, Power JD, Vincent JL et al. Modulation of the brain's functional network architecture in the transition from wake to sleep. *Prog Brain Res* 2011;193:277-294.
- (11) Spoormaker VI, Schroter MS, Gleiser PM et al. Development of a large-scale functional brain network during human non-rapid eye movement sleep. *J Neurosci* 2010;30:11379-11387.
- (12) Samann PG, Wehrle R, Hoehn D et al. Development of the brain's default mode network from wakefulness to slow wave sleep. *Cereb Cortex* 2011;21:2082-2093.
- (13) Wu CW, Liu PY, Tsai PJ et al. Variations in connectivity in the sensorimotor and default-mode networks during the first nocturnal sleep cycle. *Brain Connect* 2012;2:177-190.
- (14) Lucey BP, Bateman RJ. Amyloid- $\beta$  diurnal pattern: possible role of sleep in Alzheimer's disease pathogenesis. *Neurobiol Aging* 2014;35S2:S29-S34.
- (15) Slats D, Claassen JA, Verbeek MM, Overeem S. Reciprocal interactions between sleep, circadian rhythms and Alzheimer's disease: focus on the role of hypocretin and melatonin. *Ageing Res Rev* 2013;12:188-200.

- (16) Kang JE, Lim MM, Bateman RJ et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science* 2009;326:1005-1007.
- (17) Ooms S, Overeem S, Besse K, Rikkert MO, Verbeek M, Claassen JA. Effect of 1 Night of Total Sleep Deprivation on Cerebrospinal Fluid beta-Amyloid 42 in Healthy Middle-Aged Men: A Randomized Clinical Trial. *JAMA Neurol* 2014.
- (18) Xie L, Kang H, Xu Q et al. Sleep drives metabolite clearance from the adult brain. *Science* 2013;342:373-377.
- (19) Li J, Llano DA, Ellis T et al. Effect of human cerebrospinal fluid sampling frequency on amyloid-beta levels. *Alzheimers Dement* 2012;8:295-303.
- (20) de LL, Kilduff TS, Peyron C et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A* 1998;95:322-327.
- (21) Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature* 2005;437:1257-1263.
- (22) Kantor S, Mochizuki T, Janisiewicz AM, Clark E, Nishino S, Scammell TE. Orexin neurons are necessary for the circadian control of REM sleep. *Sleep* 2009;32:1127-1134.
- (23) Hagan JJ, Leslie RA, Patel S et al. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc Natl Acad Sci U S A* 1999;96:10911-10916.
- (24) Liguori C, Romigi A, Nuccetelli M et al. Orexinergic system dysregulation, sleep impairment, and cognitive decline in Alzheimer disease. *JAMA Neurol* 2014;71:1498-1505.
- (25) Slats D, Claassen JA, Lammers GJ, Melis RJ, Verbeek MM, Overeem S. Association between hypocretin-1 and amyloid-beta42 cerebrospinal fluid levels in Alzheimer's disease and healthy controls. *Curr Alzheimer Res* 2012;9:1119-1125.
- (26) Portelius E, Soininen H, Andreasson U et al. Exploring Alzheimer Molecular Pathology in Down's Syndrome Cerebrospinal Fluid. *Neurodegener Dis* 2014;14:98-106.
- (27) Wennstrom M, Londos E, Minthon L, Nielsen HM. Altered CSF orexin and alpha-synuclein levels in dementia patients. *J Alzheimers Dis* 2012;29:125-132.
- (28) Dauvilliers YA, Lehmann S, Jaussent I, Gabelle A. Hypocretin and brain beta-amyloid peptide interactions in cognitive disorders and narcolepsy. *Front Aging Neurosci* 2014;6:119.
- (29) Schmidt FM, Kratzsch J, Gertz HJ et al. Cerebrospinal fluid melanin-concentrating hormone (MCH) and hypocretin-1 (HCRT-1, orexin-A) in Alzheimer's disease. *PLoS One* 2013;8:e63136.
- (30) De Santi S, Pirraglia E, Barr WB et al. Robust and conventional neuropsychological norms: Diagnosis and prediction of age-related cognitive decline. *Neuropsychology* 2008;22:469-484.
- (31) Wechsler D. *Wechsler Adult Intelligence Scale--Revised*. New York: Harcourt Brace Jovanovich, 1981.
- (32) Wechsler D. *Wechsler Adult Intelligence Scale*. New York: Psychological Corporation, 1955.
- (33) Army. *Army Individual Test battery Manual of Directions and Scoring*. Washington, DC: War Department, Adjutant General's Office., 1944.

- (34) Kaplan E, Goodglass H, Weintraub S. *Boston Naming Test*. Philadelphia: Lea and Febiger, 1983.
- (35) Folstein MF, Folstein SE, McHugh PR. Mini-mental state: A practical method for grading the cognitive state of patients for the clinician. *J Psychiat Res* 1975;12:189-198.
- (36) Blennow K, Wallin A, Agren H, Spenger C, Siegfried J, Vanmechelen E. Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? *Molecular & Chemical Neuropathology* 1995;26:231-245.
- (37) Vanmechelen E, Vanderstichele H, Davidsson P et al. Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: a sandwich ELISA with a synthetic phosphopeptide for standardization. *Neuroscience Letters* 2000;285:49-52.
- (38) Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 1991;14:540-545.
- (39) Ayappa I, Norman RG, Seelall V, Rapoport DM. Validation of a self-applied unattended monitor for sleep disordered breathing. *J Clin Sleep Med* 2008;4:26-37.
- (40) Tiihonen P, Hukkanen T, Tuomilehto H, Mervaala E, Toyras J. Evaluation of a novel ambulatory device for screening of sleep apnea. *Telemed J E Health* 2009;15:283-289.
- (41) Deuschle M, Schilling C, Leweke FM et al. Hypocretin in cerebrospinal fluid is positively correlated with Tau and pTau. *Neurosci Lett* 2014;561:41-45.
- (42) Fronczek R, van GS, Frolich M et al. Hypocretin (orexin) loss in Alzheimer's disease. *Neurobiol Aging* 2012;33:1642-1650.
- (43) Kiyashchenko LI, Mileykovskiy BY, Maidment N et al. Release of hypocretin (orexin) during waking and sleep states. *J Neurosci* 2002;22:5282-5286.
- (44) Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. *Trends Neurosci* 2001;24:726-731.
- (45) Roh JH, Jiang H, Finn MB et al. Potential role of orexin and sleep modulation in the pathogenesis of Alzheimer's disease. *J Exp Med* 2014;211:2487-2496.
- (46) Scammell TE, Matheson JK, Honda M, Thannickal TC, Siegel JM. Coexistence of narcolepsy and Alzheimer's disease. *Neurobiol Aging* 2012;33:1318-1319.