1. Title Page

Reduced Slow-Wave Sleep Is Associated with High Cerebrospinal Fluid Aβ42 Levels in Cognitively Normal Elderly

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2. Abstract:

Importance: Emerging evidence suggests a role for sleep in contributing to the progression of Alzheimer disease (AD), but the features of sleep that mediate this remain poorly characterized. Slow wave sleep (SWS) is the stage during which synaptic activity is minimal and clearance of neuronal metabolites is high, making it an ideal state to regulate levels of amyloid beta (Aβ).

Objective: To examine relationships between concentrations of Aβ42 in the cerebrospinal fluid (CSF) and measures of SWS duration, slow wave activity (SWA) and SWS continuity in cognitively normal elderly subjects.

Design: We analyzed Aβ42, P-Tau and, T-Tau in CSF by ELISA. Nocturnal polysomnography (NPSG) was performed within 6.7±7.5 months of the lumbar puncture and analyzed for SWS duration, total SWA, SWA in NREM cycles 1-4, and mean SWS bout length.

Setting: Community dwelling elderly subjects were evaluated at the NYU Center for Brain Health and received NPSG's at the NYU Sleep Disorders Center.

Participants: 36 elderly subjects (age 66.8±8.2) with normal cognition and without significant sleep disordered breathing

Main Outcome and Measure: Correlations and linear regression analyses were used to assess for associations between CSF Aβ42 levels and measures of SWS controlling for potential confounders. Resulting models were compared to each other using ordinary least squared linear regression analysis. Additionally, the participant sample was dichotomized into 'high' and 'low Aβ42' groups to compare SWS bout length using survival analyses.

Results: Total sleep time, time spent in NREM1, NREM2 or REM was not correlated with CSF Aβ42. A significant inverse correlation was found between CSF Aβ42 levels, SWS duration and other SWS characteristics. Collectively, total SWA in the frontal lead was the best predictor of reduced CSF Aβ42 levels when controlling for age and ApoE4 status.

Conclusion and Relevance: In cognitively normal elderly, reduced and fragmented SWS is associated with increases in CSF Aβ42.

3. Text

INTRODUCTION:

The 'Amyloid Cascade Hypothesis' posits that the deposition of amyloid beta (Aβ) in the brain is the initiating pathological event in Alzheimer's disease (AD) (1). Several studies have provided evidence that Aβ dynamics are influenced by the sleep-wake cycle. In transgenic mice, soluble Aβ levels are higher in the interstitial fluid during wakefulness and lower during sleep, while sleep deprivation increases Aβ concentrations and accelerates plaque deposition (2). In humans, cerebrospinal fluid (CSF) Aβ42 also exhibits a diurnal pattern, with the lowest levels occurring in the morning sampling (3). This physiological morning decrease in CSF Aβ42 is abolished by total sleep deprivation (4). All these findings suggest that sleep may play a unique role in AD by resetting soluble Aβ42 to lower levels, however, the precise regulation of this diurnal pattern is not well understood.

Aβ production is thought to be neuronal activity-dependent, and plaque deposition preferentially targets brain regions with high neuronal and large-scale synchronous activity (5). During sleep, the brain remains predominantly active with preservation of cortico-cortical connectivity during light sleep, *i.e.* non-REM (NREM) sleep stages 1-2. However, there is a reduction in fronto-parietal connectivity that occurs with increasing depth of sleep, to the point of being significantly reduced at deep sleep, *i.e.* slow wave sleep (SWS) or NREM stage 3 (NREM3) (6). During REM sleep, brain activation becomes more frequent (7). In this study, we assessed the effect of SWS characteristics on morning CSF Aβ42 levels in a group of cognitively normal elderly. We hypothesized that conserved SWS would be associated with low morning CSF Aβ42 levels, while disrupted SWS would be associated with high morning Aβ42 levels. Given that reduced CSF Aβ42 levels and SWS duration can be associated with advanced age, the ApoE4 allele, male gender and lower number of years of education, we also tested the extent to which the variation in CSF Aβ42 predicted by SWS were influenced by these factors.

METHODS:

Study Design and Participants

Among a pool of elderly participating in NIH-supported longitudinal studies, this study included a sub-set of 41 subjects that agreed to undergo nocturnal polysomnography (NPSG). Subjects were recruited from multiple community sources in NYC as previously described (8). Individuals with medical conditions that could affect brain structure or function were excluded.

Procedures

Subjects received a clinical standardized diagnostic assessment part of the Uniform Data Set II at the NYU Center for Brain Health (9). In addition, subjects had laboratory examinations and underwent a structural MRI, a morning lumbar puncture (LP) and a NPSG. All subjects were diagnosed as cognitively normal (Clinical Dementia Rating [CDR]=0). ApoE4 genotype was determined using the Polymerase Chain Reaction (11). CSF samples were processed as described previously (8). CSF was analyzed for Aβ42, phosphorylated tau at threonine$_{181}$ (P-tau) and total tau (T-Tau) using ELISA kits (Fujirebo, Ghent, Belgium)(12).

All subjects received structural volumetric MRI scans as part of the parent NIH grants on a 1.5T (GE, USA) or 3T (Siemens, Germany) system using standardized procedures (13;14). These scans were obtained to rule out MRI evidence of intracranial mass and white matter disease prior to performing the LPs. Age-related atrophy in the medial prefrontal cortex (mPFC) cortex has been associated with reduced slow wave activity (SWA) in older adults (15). Because atrophy mediates SWS disruption, we measured cortical volumes from MPRAGE sequences using the FreeSurfer toolkit (16). We then computed gray matter volumes to create an mPFC region of interest (ROI) using the following bilateral ROIs: caudal anterior cingulate cortex, medial orbitofrontal cortex, rostral anterior cingulate cortex and superior frontal gyrus (23). Resulting ROIs were adjusted (residualized) to their intracranial volume using linear regression.

Sleep recordings were performed overnight at the NYUSDC as previously described (17). They consisted of six EEG channels (F3, F4, C3, C4, O1 and O2), two electro-oculographic (EOG) leads and one chin electromyographic channel. Visual scoring of recordings, total sleep time (TST) and sleep stage duration in minutes were determined according to AASM criteria (17). Respiratory events were scored using AASM criteria as described previously (8). AHI4% was defined as the sum of all apneas and hypopneas with ≥4% desaturation divided by TST in hours. AHI-all was defined as the sum of apneas and hypopneas (3% or arousal) divided by TST in hours.

Sleep studies were first scored in 30-second epochs (17). NREM-REM cycles were defined according to the criteria of Feinberg and Floyd (18) starting with NREM2 and containing at least 15 minutes of NREM2 or NREM3 followed by a REM episode of at least 5 minutes. EEG signals, acquired with a sampling frequency of 256Hz, were then segmented into 5-second epochs. Power spectra of artifact-free epochs were computed using the Fast-Fourier Transform and matched with the 5-second sleep scores. SWA was calculated using the average power density in the 0.5-4.0 Hz range of F4, C3 and O2 full-night EEG recordings. Changes in SWA were evaluated using areas under

the curve (AUC) for each NREM sleep cycle and for the full night. To account for individual differences in the occurrence and duration of sleep cycles, NREM episodes were first subdivided into 24 equal segments, and then averaged in each segment (19). Where appropriate, group comparisons of SWS characteristics were performed using the median CSF Aβ42 value to divide the sample into two equal sized ('high' and 'low') Aβ42 groups.
Outcome measures used in statistical analyses were age, gender, ApoE4 status, years of education, CSF biomarkers (Aβ42, P-Tau, T-Tau), SWS duration, percent of TST spent in SWS (%SWS), SWA full night, mean SWS bout length, SWA in NREM cycles 1-4 and mPFC.

Statistical Analyses

Logarithm transformation was applied to normalize right skewed variables (CSF Aβ42, SWA in F4, C3 and O2) prior to analysis. We first assessed the effect of SWS duration and other SWS characteristics on morning CSF Aβ42 levels in the entire group using correlation analyses. We then used ordinary least squared (OLS) linear regression to evaluate the associations between the SWS characteristic with the highest correlation coefficient and CSF Aβ42, using Aβ42 as the dependent variable. Age, ApoE4 status, sex and years of education were included as covariates only if they improved the R^2 and adjusted R^2 for the model. The best fitting model was then replicated with each of the other SWS characteristics. On a final step we compared the resulting models looking at percent increase in R^2 for each model.
Finally, we analyzed SWS bout length after dichotomizing the sample into 'low Aβ42' and 'high Aβ42' groups using the median CSF Aβ42 (536.9 pg/ml). Survival analysis was used to estimate the time of interest in the duration from the onset of sleep (or a specific stage thereof) to the transition to wake (or to some other stage). The period between the onset of sleep and a terminating transition was used to define a 'run'. 'SWS runs' were defined as consecutive epochs of SWS bounded by either NREM1, NREM2, REM or wake. Using the standard techniques of survival analysis, a bootstrap-based analysis that accounted for the number of runs contributed by each subject was performed to determine a cumulative duration probability distribution for SWS. In order to remain consistent across all subjects and, at the same time, retain a sufficient number of data points, SWS runs were randomly sampled up to the median number in the cohort. Log rank tests were used to compare both groups on the curves derived from the sampling procedure. This procedure was repeated 1,000 times. At each step of the iteration, a p

value was estimated from the history of prior p values, yielding an increasingly stable result as the number of iterations increased.

RESULTS:

Healthy, cognitively normal group with low overall risk for AD:

41 eligible participants completed all study procedures. Five were excluded due to moderate to severe SDB (AHI4%≥15) (3 subjects), fragmented sleep with TST <3 hours (1 subject) and alcohol consumption prior to the NPSG (1 subject). Demographic, cognitive, and health characteristics of the remaining 36 participants are shown in Table 1 ('Global'). Overall, it was a sample of mostly non-obese (BMI 25.8±3.7 kg/m^2), highly educated (16.3±2.1 years of education), elderly (age 66.9±8.3 years), in good general health. Table 1 displays mean values of CSF Aβ42, T-Tau, and P-Tau levels. Overall, it was a healthy group with low overall risk for AD.

Sleep characteristics:

Table 3 summarizes sleep architecture characteristics. Only 5 subjects had mild SDB (AHI4%=5-14.99), while the majority of subjects had normal breathing during sleep (AHI4% <5). Epworth sleepiness scores did not suggest the presence of daytime sleepiness in our sample (value 6.4±3.9). Table 2 shows the SWS characteristics of our sample. SWS duration was inversely correlated with age ($r=-0.36$, $p<0.05$) and WASO ($r=-0.51$, $p<0.05$), but was not associated with BMI; AHI4%, AHIall, mean O_2Sat during sleep, TST, NREM1, NREM2 or REM duration; or with mPFC volume. As expected (21;22), SWS duration was higher in females than in males even after controlling for age and WASO ($F_{1\text{-}33}=4.5$, $p<0.05$; females: 76.8±27.1 min, males: 47.4±30.2 min).

Effects of SWS duration and power on CSF Aβ42 levels:

We first examined whether SWS duration correlated with CSF Aβ42 levels. There was a significant inverse correlation between CSF Aβ42 and SWS duration ($r=-0.35$, $p<0.05$) (Figure 1), %SWS ($r=-0.36$, $p<0.05$), total SWA in F4 ($r=-0.45$, $p<0.01$) (Figure 1), SWA in cycle 1 in F4 ($r=-0.41$, $p<0.05$), and SWA in cycle 2 in F4 ($r=-0.38$, $p<0.05$). Similar but weaker inverse correlations were found between CSF Aβ42 and SWA in the C3 channels (Table 4) and there were no associations with SWA in the O2 channels. CSF Aβ42 was not correlated with the duration of other sleep stages. Using OLS, the best prediction model for CSF Aβ42 included total SWA in F4, sex

and ApoE4 status (Table 3). Based on % increase in R^2, total SWA in F4 reduced the variation in Aβ42 by 116.98%, compared to the model that only included sex and ApoE4. The next best sleep predictors for CSF Aβ42 were SWA in cycle 1 in F4, followed by SWA in cycle 2 in F4, which increased the R^2 by 98.11% and 69.81%, respectively (Table 3).

Effects of SWS continuity in 'low Aβ42' and 'high Aβ42' groups on CSF Aβ42 levels:

There were no clinical differences between 'high' and 'low' Aβ42 groups except for age, which was lower in the 'low Aβ42' ($F_{1\text{-}35}$=4.5, p<0.05; 'high Aβ42' group: 70.2±9.0 years, 'low Aβ42' group: 63.7±6.3years) (Table 1). The cumulative duration probability distribution for SWS was significantly left-shifted in the 'high Aβ42' compared to 'low Aβ42' subjects (p <0.01), indicating that SWS was more fragmented and occurred in shorter bouts in 'high Aβ42' subjects than in the 'low Aβ42' group. Conversely, the cumulative duration probability distribution for NREM2 was significantly right-shifted in the 'high Aβ42' compared to 'low Aβ42' subjects (p <0.01), indicating NREM2 sleep was less fragmented and occurred in longer bouts in 'high Aβ42' subjects. There were no significant differences in the duration probability distribution of NREM1 or REM sleep between groups.

While the cumulative duration probability distribution reflects sleep continuity across groups, individual measures of sleep stage continuity can be represented by stage mean bout length (with longer length reflecting increased sleep consolidation) and percent of runs of sleep lasting less than 3 minutes (with a higher percentage reflecting decreased sleep consolidation). Based on the results of the survival curve analysis, we examined the correlation between CSF Aβ42 levels and measures of NREM2 and SWS continuity. Across all subjects, there were no significant correlations between CSF Aβ42 levels and NREM2 continuity variables. On the other hand, we observed a significant inverse correlation between CSF Aβ42 levels and SWS mean bout length (rho=-0.37, p <0.05) and a significant positive correlation between CSF Aβ42 levels and percent of runs of SWS less than 3 minutes (rho=0.42, p=0.01). Using a partial correlation to control for age, we showed a continued significant positive partial correlation between CSF Aβ42 levels and percent of runs of SWS less than 3 minutes (rho=0.36, p <0.05), suggesting that controlling for age had little effect on the strength of the relationship between these variables. A partial correlation controlling for age demonstrated a reduced strength of association between CSF Aβ42 levels and SWS mean bout length (rho=-0.31, p=0.069), suggesting that age has some mediating effect on this relationship.

DISCUSSION:

Understanding the relation between sleep and Aβ might present important opportunities for therapy to slow the progression of AD. Although soluble Aβ42 levels may be influenced by several factors in the elderly, changes in sleep common in late-life such as age-dependent loss of SWS and increased incidence of insomnia and sleep apnea (24) could lead to relative high brain soluble Aβ42 levels in the stages prior to amyloid deposition. Our findings provide a link between diminished SWS duration, continuity, and delta power with high CSF Aβ42 in a normal aging group.

Evidence from both human and animal models suggests that Aβ production is neuronal activity-dependent, following a diurnal pattern wherein peak levels occur during periods of activity and decline during sleep (12-14). Aβ production is thus postulated to decrease predominantly during SWS due to the decreased neuronal activity observed in this sleep stage. In view of our results, a decrease in CSF Aβ42 would occur in periods of sleep with high SWA in the frontal lead, although these relationships were also observed in the central electrodes and were present in all NREM sleep cycles. Our current observations are consistent with previous human studies in which nadirs in lumbar CSF Aβ42 levels occurred at a point roughly 2/3 of the way through typical TST, after which most SWS has occurred and after which sleep is predominated by stages NREM1-2 and REM (3). Attenuation of the Aβ diurnal pattern with age may be explained by a relative increase in neuronal activity following disturbed sleep, reflecting age-related loss of SWS, which in turn may elevate Aβ levels and promote local amyloid deposition. Synaptic downscaling during sleep is thought to be necessary to counter waking activity synaptic potentiation and associated growth, which would otherwise exceed available resources of energy and space (25). This synaptic homeostasis theory proposes that most downscaling is achieved during SWS. Given that synaptic activity is thought to increase CSF Aβ concentrations, and SWS is a stage of sleep where there is a decrease in brain connectivity and a global downscaling, SWS may therefore be the stage that is most responsible for the morning after sleep decreases in CSF Aβ42 (26). An additional possible mechanism involves sleep's putative role in the clearance of brain metabolites, including Aβ (27). Fragmented SWS would reduce egress of Aβ out of the brain, leaving higher concentrations in the brain interstitial fluid that is ultimately reflected in CSF concentrations.

It bears noting that the functional significance of elevated CSF Aβ42 levels is not established. Although it makes intuitive sense that higher concentrations of CSF Aβ42 would foster its aggregation, longitudinal studies of how CSF concentrations of Aβ42 change over time, particularly as cognitively normal subjects progress to dementia, has

not been carefully studied. While mouse models show early increases in Aβ before late decreases (28), there is only incidental cross-sectional evidence of elevations in CSF Aβ42 in human studies from familial AD mutation carriers (29) and cognitively normal elderly in early pre-symptomatic stages of the disease (30;31) . While it has been demonstrated that CSF levels of Aβ42 are about 50% of control levels when compared to age-matched subjects without AD (32), it remains to be determined whether there is a period of elevated CSF Aβ42 in humans prior to decline as our data suggests.

The interval between polysomnography and CSF collection was of 6.7±7.5 months. Although neither SWS nor CSF Aβ42 change markedly over this short duration in normal subjects, we nonetheless recognize this may have introduced variability. Additionally, measurement of SWS itself may have been affected by the equipment required for its recording. However, most subjects completed home sleep monitoring as part of existing studies prior to in-lab polysomnography such that some level of acclimation to the recording equipment was likely.

Our results cannot define the causal relationship between reduced SWS and high CSF Aβ42. Although we favor the model in which age-dependent long term disruption of SWS promotes higher Aβ42, disturbed sleep may alternatively be a consequence of accumulated extracellular Aβ42 early in the progression of AD pathology rather than a key event in AD pathogenesis. The sleep-wake changes described in APP-PS1 mice may be due to induced changes in synaptic activity and excitability occurring in brain regions affected by amyloid deposition (33). In humans, a recent study found that individuals diagnosed with AD had fewer neurons than controls in the intermediate nucleus, a brain region that is thought to promote sleep by inhibiting wake-promoting brain regions (34). Additionally, the impairment in sleep-dependent declarative memory consolidation observed in subjects with high amyloid load was found to be mediated by the loss in frontal SWA (35), suggesting that changes in SWS lie downstream of Aβ42 deposition. Because these observations are not mutually exclusive of an effect of SWS on soluble Aβ42, the interaction between a loss of SWS and Aβ42 may perpetuate a positive feedforward cycle. Amyloid deposition may damage neurons responsible for generating slow waves, further disturbing sleep and elevating Aβ42 levels during wakefulness (13) until a certain degree of amyloid burden is reached that captures and prevents the transport of soluble Aβ42 from the brain to the lumbar space CSF (36).

Irrespective of an effect of Aβ42 on sleep, the effect of SWS on soluble Aβ42 is noteworthy because it raises the possibility for new disease-modifying drug targets that slow AD progression. The mechanism by which SWS can be modified may take many forms. In older subjects with SDB, treatment with CPAP improves sleep architecture

including increased SWS (37). In older subjects without SDB, transcranial magnetic stimulation (38) and existing medications (39;40) may increase or trigger SWS and reduce Aβ42 production. Whether such interventions in older subjects affect Aβ42 metabolism and/or progression of clinical AD remains to be tested, but our current findings support further investigation.

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6. Figure Legends and Tables

Table 1. Sociodemographic, Clinical and CSF Data of Study Group

	Global (n=36)	High Aβ42 (n=18)	Low Aβ42 (n=18)	P-Value High vs. Low Aβ42 group
Age, mean (SD), years	66.8 (8.2)	69.9 (8.6)	63.6 (6.5)	0.02
Male Sex, No. (%)	17(47.2%)	11(61.1%)	6 (33.3%)	0.10
BMI, mean (SD), kg/m^2	25.8 (3.7)	26.3 (3.6)	25.2 (3.8)	0.39
Education, mean (SD), years	16.3 (2.1)	16.4 (2.5)	16.2 (1.7)	0.82
Global Clinical Dementia Rating (CDR)	0	0	0	
MMSE, mean (SD)	29.3 (1.1)	29.1 (1.1)	29.4 (1.1)	0.46
Hypertension, No. (%)	11(27.8)	7(38.9)	4(22.2)	0.28
Cardiovascular disease, No. (%)	1(2.8)	1(5.6)	0(0)	0.31
Diabetes, No. (%)	1(2.8)	1(5.6)	0(0)	0.31
Thyroid, No. (%)	7(19.4)	3(16.7)	4(22.2)	0.67
ApoE4+, No**. (%)**	11(30.6)	4(22.2)	7(38.9)	0.28
Ethnicity (White, Hispanic, African American, Asian), No. (%)	26 (72.2), 2(5.6), 7(19.4), 1(2.8)	16(88.9), 0(0), 2(11.1), 0(0)	10(55.6), 2(11.1), 5(27.8), 1(5.6)	
Aβ42, median (IQR), pg/mL	539.7 (269.8)	729.8 (359.3)	474.8 (108.9)	p<0.001
P-Tau, median (IQR), pg/mL	38.7 (19.3)	40.8 (14.5)	33.2 (17.7)	0.27
T-Tau, median (IQR), pg/mL	232.0 (13.4)	260.0 (158.9)	189.7 (140.0)	0.10

Table 2. Slow Wave Sleep Characteristics of Study Group

	Global	High Aβ42	Low Aβ42	P-value
SWS, mean (SD), min	62.9 (31.9)	50.3 (27.7)	75.5 (31.4)	0.12
%SWS, mean (SD)	17.4 (8.8)	13.5 (7.2)	21.4 (8.7)	0.05
Mean N3 runs, median (IQR)		1.8 (1.1)	2.4 (1.5)	0.04*
% N3 runs ≤ 3min, median (IQR)		51.4 (47.5)	32.2 (18.1)	0.02*
% N3 runs ≥ 5min, median (IQR)		37.9 (57.9)	59.9 (22)	0.03*

Table 3. Linear Regression Analysis

Dependent	Independent	B	t	Pr > |t|	R2	Adjusted R2	% Increase R2	F	p
LnAβ42	Intercept	6.68	36.67	<0.01	0.106	0.05	n/a	1.96	0.2
	ApoE4 status	-0.15	-1.24	0.22					
	sex	-0.17	-1.11	0.14					
	LnSWAfullnightF4	-0.38	-2.25	0.03	0.23	0.16	116.98	3.16	0.04
	LnSWAcycle1F4	-0.40	-2.09	0.05	0.21	0.14	98.11	2.89	0.05
	LnSWAcycle2F4	-0.30	-1.75	0.09	0.18	0.10	69.81	2.41	0.09
	LnSWAfullnightC3	-0.28	-1.56	0.13	0.17	0.09	60.38	2.17	0.11
	LnSWAcycle1C3	-0.27	-1.35	0.19	0.15	0.08	45.28	1.95	0.14
	LnSWAcycle2C3	-0.24	-1.34	0.19	0.15	0.07	45.28	1.93	0.14
	% Time SWS	-0.26	-1.33	0.19	0.15	0.07	44.34	1.92	0.15
	SWS Duration	-0.25	-1.32	0.20	0.15	0.07	43.40	1.91	0.15

Table 4. Pearson Correlation with LnAβ42

	R	P-Value
LnSWAfullnight_F4	-0.45	0.005
LnSWAcycle1_F4	-0.41	0.01
LnSWAcycle2_F4	-0.38	0.02
LnSWAfullnight_C3	-0.38	0.02
Percent time spent in SWS	-0.36	0.03
SWS duration	-0.35	0.03
LnSWAcycle1_C3	-0.34	0.04
LnSWAcycle2_C3	-0.34	0.04
ApoE4 status	-0.23	0.18
Sex	-0.25	0.14
Age	0.23	0.16
Years of education	-0.14	0.42

Figure 1. Scatter Plots of Natural Log of Aβ42 and F4 Slow Wave Activity (Full Night) and Slow Wave Sleep Duration