Study Objectives: Growing evidence suggests that sleep disturbances precede by years the clinical onset of Alzheimer disease (AD). The goal of the current study is to determine whether changes in polysomnographic (PSG) sleep patterns accompany subjective sleep complaints in patients with mild cognitive impairment (MCI). We further examine whether meaningful changes in objective sleep physiology are predicted by self-reported sleep measures in MCI patients, and whether incipient neurodegeneration contributes to exacerbate sleep misperception.

Design, Setting, and Participants: Overnight PSG recordings and self-reported sleep measures were obtained from 25 healthy elderly (HE) subjects and 25 patients with MCI at the sleep laboratory.

Results: Both PSG and self-reported sleep measures confirmed that sleep is altered in patients with MCI. Whereas subjective sleep responses predicted fragmentation of slow wave sleep (SWS) in HE individuals, this relationship was not evident in MCI patients. Furthermore, patients with MCI showed significant discrepancies in the estimation of sleep onset latency when compared with HE subjects.

Conclusions: Sleep is significantly impaired in patients with mild cognitive impairment at both the objective and subjective level, which may be used as a surrogate marker of preclinical Alzheimer disease. Taken together, these findings aid in the development of novel therapeutic strategies devoted to improve sleep in the elderly population at risk of developing Alzheimer disease.

Keywords: Aging, Alzheimer disease, ApoE, mild cognitive impairment, polysomnography, self-reports, sleep, sleep misperception

Citation: Hita-Yañez E; Atienza M; Cantero JL. Polysomnographic and subjective sleep markers of mild cognitive impairment. SLEEP 2013;36(9):1327-1334.
METHODS

Patients

Twenty-five patients with MCI (7 females, mean age: 70.5 ± 6.8 y) and 25 HE subjects (13 females, mean age: 67.1 ± 5.3 y) were enrolled in the study, after they signed an informed consent. Experimental procedures were previously approved by the Ethical Committee for Human Research at the University Pablo de Olavide. Demographic and cognitive profiles of the participant groups are shown in Table 1.

Both HE subjects and patients with MCI underwent a neurological examination to exclude potential neurological diseases. Cerebral magnetic resonance imaging (MRI) was also performed on all candidates to rule out lesions such as territorial cerebral infarction, brain tumor, hippocampal sclerosis, and/or vascular malformations. Those candidates who showed periventricular and/or deep white matter damage, derived from scorings ≥ 2 on the Fazekas ischemic scale, were not included in the study. Other exclusion criteria were a history of neurological conditions, psychiatric disorders, and/or major medical illness (chronic renal, hepatic, pulmonary, or endocrine), the use of medication affecting the sleep-wake cycle (benzodiazepines, tricyclic and/or seroton reuptake inhibitors), the presence of depressive symptoms (assessed with the abbreviated version of the Geriatric Depression Scale, using 5 as a cutoff score), and/or having complaints of sleep disordered breathing, movement disorders during sleep or unusual sleep schedules (i.e., shift work), which was corroborated by bed partners and/or caregivers. Patients with MCI were not taking cholinesterase inhibitors at the time of recruiting.

The diagnosis of MCI was based on consensus criteria: (1) subjective memory complaints corroborated by the informant, (2) objective memory loss substantiated by neuropsychological tests (scorings 1.5 standard deviations below the age-appropriate mean; immediate and delayed recall were assessed by the Spanish version of the Logical Memory subtest contained in the Wechsler Memory Scale—Third Edition), (3) global score of 0.5 (questionable dementia) in the clinical dementia rating (CDR), (4) normal independence function, and (5) not meeting the Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition (DSM-IV) criteria for dementia. Global cognitive status was assessed with the Mini Mental State Examination (MMSE). Inclusion criteria for HE subjects were absence of objective memory deterioration as revealed by the same neuropsychological tests used with MCI patients, CDR global score of 0 (no dementia), and normal independent function.

ApoE Genotyping

Genomic DNA was isolated from 3 mL human whole blood using a standard salting-out protocol. ApoE polymorphisms were determined with polymerase chain reaction (Step-One Plus, Applied Biosystems, USA) using predesigned TaqMan single nucleotide polymorphisms genotyping assays (Applied Biosystems, USA).

PSG Recordings

The PSG protocol included simultaneous recordings of electroencephalography (EEG), vertical and horizontal electrooculography, and electromyography of submental muscles. Electrophysiological recordings were performed with gold cup, 10 mm- diameter electrodes (Grass, USA) filled with electrolytic cream, and attached with surgical tape (face placements) and collodion (scalp placements). Overnight PSG recordings were performed in a sound-attenuated bedroom with infrared video-controlled supervision. Respiratory measures were not included in the protocol because none of the participants reported complaints of sleep disordered breathing, corroborated by their bed partners. Furthermore, scores of the Epworth Sleepiness Scale (ESS) were below the cutoff for suspected sleep disorders associated with excessive daytime sleepiness.

Electrophysiological recordings were amplified (BrainAmp MR, Brain Products, Germany), filtered (0.1-100 Hz bandpass), digitized (250 Hz, 16-bit resolution), and stored in digital format for off-line analysis. A trained technician, blind to the study purpose, conducted scoring of sleep stages following standard criteria. Criteria for scoring EEG arousals were taken from the American Sleep Disorders Association report, and the level of sleep fragmentation was determined by the arousal index in each sleep stage. This index resulted from dividing the number of arousals appearing in a sleep stage by the time (in hours) spent in that sleep stage. For the purpose of the current study, only those PSG parameters that showed significant group differences between HE subjects and patients with MCI (i.e., REM percentage and density of SWS arousals) were correlated with self-reported sleep data.

<table>
<thead>
<tr>
<th>Table 1—Demographic characteristics and cognitive profile</th>
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<tbody>
<tr>
<td>HE (n = 25)</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Sex (F/M)</td>
</tr>
<tr>
<td>Education level</td>
</tr>
<tr>
<td>CDR (sum of boxes)</td>
</tr>
<tr>
<td>MMSE</td>
</tr>
<tr>
<td>Immediate recall</td>
</tr>
<tr>
<td>Delayed recall</td>
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<tr>
<td>Forgetting rate</td>
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</table>

The forgetting rate was obtained by subtracting scores of immediate memory from delayed memory. Results expressed as mean ± standard deviation. CDR = 0 no dementia, CDR = 0.5 questionable or very mild dementia. MMSE (Mini Mental State Examination) scores ranged from 0 to 30. N/A (not applicable). ε4−, ApoE ε4 non-carriers; ε4+, ApoE ε4 carriers; CDR, Clinical Dementia Rating; F/M, female/male; HE, healthy elderly; MCI, mild cognitive impairment.
Subjective Sleep Data

Subjective sleep measures were collected the same day from the PSG recording through direct interviewing. All participants were asked to answer five questions focused on sleep parameters, sleep symptoms, and sleep quality over the past few months (Table 2). These five questions were included in the current study on the basis of sleep disturbances previously reported in patients with AD or MCI: longer latencies to sleep onset\(^1\) (item 1), shortened sleep duration\(^2\) (item 2), increased sleep arousals and/or wake ups after sleep onset\(^3,4,26\) (items 3 and 4), and poorer sleep quality\(^1\) (item 5).

Statistical Analysis

Statistical analyses were conducted using SPSS v.15 (SPSS Inc., Chicago, IL). A logarithmic transformation was applied to non-normally distributed dependent variables to approach normal distribution. Group differences in sex (HE versus MCI; MCI ε4 noncarriers versus MCI ε4 carriers) were evaluated by applying the chi-square test, whereas group differences in the remaining demographic and cognitive variables were assessed with the Student \(t\)-test.

Group differences in PSG and subjective sleep measures were separately assessed by analyses of covariance (ANCOVA). Next, multivariate regression (MVAR) analyses were applied to examine if self-reported sleep predicted group differences in PSG parameters. If statistical significance was reached in at least one group, we then assessed group differences among the three groups (HE, MCI, MCI ε4 noncarriers, MCI ε4 carriers). If significance was reached in at least one group, we then assessed group differences between regression slopes. Both ANCOVAs and MVAR analyses (within and between groups) included age and sex as covariates.

Given the relationship between memory consolidation and sleep continuity,\(^27\) we further evaluated whether any sleep parameter, derived from either overnight PSG recordings or self-reports, predicted memory performance (immediate, delayed memory, and forgetting rate) in each group separately (HE, MCI, MCI ε4 noncarriers, MCI ε4 carriers). If significance was reached in at least one group, we then assessed group differences between regression slopes. Both ANCOVAs and MVAR analyses (within and between groups) included age and sex as covariates.

We finally evaluated whether sleep misperception gains relevance during prodromal AD stages. To achieve this goal, two mixed ANCOVAs were performed with either sleep onset latency (SOL) or sleep duration (subjective versus objective) as the within-subject factor, group (either HE versus MCI or MCI ε4 noncarriers versus MCI ε4 carriers) as the between-subject factor, and age and sex as covariates.

RESULTS

Demographic and Cognitive Profile

HE subjects and patients with MCI showed similar demographic profiles (Table 1). Age was comparable in the two groups but differed between MCI ε4 carriers and MCI ε4 noncarriers (\(P < 0.01\)). Although sex did not differ between HE and MCI, this variable showed a trend toward significance (\(P < 0.08\)). Eleven patients with MCI exhibited the genotype ApoE ε4 and the remaining 14 patients were ε4 noncarriers. Overall, the prevalence of the allele ε4 in the ApoE was 3.6-fold greater in MCI than in HE, being present in 44% of MCI patients in contrast with 12% of HE subjects. The presence of the ApoE ε4 allele was not used as selection criterion during the recruiting process, its distribution in our MCI sample resulted entirely from chance.

Patients with MCI showed impairments in immediate (\(P < 10^{-6}\)) and delayed recall (\(P < 10^{-5}\)), as well as in the forgetting rate (immediate minus delayed recall; \(P < 0.006\)) when compared with HE subjects (Table 1). In addition, memory, but not the forgetting rate, was significantly more affected in MCI ε4 carriers than in noncarriers (immediate, \(P < 0.008\); delayed, \(P < 0.002\)). The same pattern of results (HE > MCI-ε4 > MCI-ε4\(^+\)) was corroborated when memory performance was compared among the three groups (Pillai trace, \(F_{4,94} = 10.6, P < 10^{-6}\); post hoc analyses, \(P < 0.03\)).

PSG Sleep

Group differences in PSG sleep variables were reported elsewhere.\(^14\) Here, statistical analyses were repeated introducing age and sex as covariates into the general linear model, although

Table 2—Self-reported sleep questions

1. How long does it usually take you to fall asleep? ______ min
2. How long do you sleep at night? ______ h
3. How many times do you awaken per night? ______ times
4. Do you have difficulty in returning to sleep after nocturnal awakenings?
   Usually (three or more times a week)
   Sometimes (once or twice a week)
   seldom (less than once a week)
   Never
5. Rate your sleep quality from 0-10 (0 being terrible, 10 being excellent).

Table 3—Self-reported sleep in HE subjects and patients with MCI

<table>
<thead>
<tr>
<th>Sleep Self-Report</th>
<th>HE (n = 25)</th>
<th>MCI (n = 25)</th>
<th>P &lt;</th>
<th>MCI ε4(^-) (n = 14)</th>
<th>MCI ε4(^+) (n = 11)</th>
<th>P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sleep latency (min)</td>
<td>19.2 ± 17.1</td>
<td>34.3 ± 28.13</td>
<td>0.05</td>
<td>29.5 ± 22</td>
<td>40.5 ± 33.7</td>
<td>0.7</td>
</tr>
<tr>
<td>2. Sleep time (h)</td>
<td>6.4 ± 0.74</td>
<td>5.94 ± 1.25</td>
<td>0.01</td>
<td>5.78 ± 1.19</td>
<td>6.15 ± 1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>3. Nocturnal awakenings</td>
<td>1.22 ± 1.3</td>
<td>2.36 ± 1.53</td>
<td>0.01</td>
<td>2.35 ± 1.75</td>
<td>2.36 ± 1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>4. Sleep after awakenings</td>
<td>1.6 ± 0.63</td>
<td>2.48 ± 1.17</td>
<td>0.0004</td>
<td>2.35 ± 1.1</td>
<td>2.63 ± 1.22</td>
<td>0.7</td>
</tr>
<tr>
<td>5. Sleep quality (0-10)</td>
<td>7.56 ± 1.16</td>
<td>6.48 ± 1.7</td>
<td>0.003</td>
<td>6.78 ± 1.77</td>
<td>6.1 ± 1.5</td>
<td>0.1</td>
</tr>
<tr>
<td>ESS</td>
<td>5 ± 2.4</td>
<td>5.4 ± 3.5</td>
<td>0.6</td>
<td>5.1 ± 2.6</td>
<td>5.8 ± 4.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation. Epworth Sleepiness Scale (ESS) scores ranged from 0 to 24, 10 being the cutoff to suspect pathological diurnal sleepiness. ε4\(^-\), ApoE ε4 non-carriers; ε4\(^+\), ApoE ε4 carriers; HE, healthy elderly; MCI, mild cognitive impairment.
results remained unchanged. Briefly, SWS was significantly disrupted in patients with MCI as revealed by the higher density of arousals occurring during this cerebral state (HE = 0.09 ± 0.11; MCI = 0.19 ± 0.10; P < 0.01). REM sleep was also significantly shortened in patients with MCI (HE = 14.7 ± 3.7; MCI = 10.1 ± 5.4; P < 0.007). This effect was especially evident in MCI ε4 carriers (7.4 ± 5.5; P < 0.04) when compared with MCI ε4 noncarriers (12.3 ± 4.3).

Self-Reported Sleep

Overall, self-perception of sleep was worse in patients with MCI than in HE individulas (Pillai trace, F5,42 = 5.37, P < 0.001). Post hoc analyses showed that MCI patients reported longer SOL (F1,46 = 3.92, P < 0.05), shorter sleep time (F1,46 = 6.02, P < 0.01), increased nocturnal awakenings (F1,46 = 6.97, P < 0.01), more difficulty in sleeping after nocturnal awakenings (F1,46 = 14.34, P < 0.0004), and poorer sleep quality (F1,46 = 10.08, P < 0.003).

No differences in subjective sleep were found when compared responses between MCI ε4 carriers and noncarriers. Table 3 summarizes averaged group responses to the sleep questions employed in this study. Subjective levels of daytime sleepiness did not differ between groups, as revealed by ESS scores (Table 3).

Relationships Between Sleep Physiology and Self-Reports of Sleep

We further investigated whether significant group differences in meaningful PSG sleep parameters (SWS arousals and REM percentage) correlated with responses to the five sleep items in HE subjects and patients with MCI, separately. The regression analysis yielded a positive relationship between SWS arousals and self-reported sleep in HE subjects (F2,24 = 3.1, P < 0.02, adjusted R squared = 0.38), but no significant associations between objective and subjective sleep were found in MCI patients. Post hoc analyses revealed that two sleep items mainly accounted for significant correlations between SWS arousals and self-estimation of sleep disturbances in HE subjects: “difficulty in sleeping after nocturnal awakenings” (P < 0.03, r = 0.4) and “number of nocturnal awakenings” (P < 0.04, r = 0.37). However, only correlations performed with “difficulty in sleeping after awakenings” significantly distinguished normal from pathological aging (F5,40 = 4.32, P < 0.003, adjusted R squared = 0.25; beta for the interaction term = 0.64, P < 0.03), although correlations with “number of nocturnal awakenings” also showed a trend toward significance (P < 0.07). Figure 1 illustrates between-group regression analyses for the abovementioned relationships. Neither responses to sleep questions nor the ApoE ε4 polymorphism predicted the amount of REM sleep in HE subjects and patients with MCI.

Relationships Between Sleep and Memory Performance

Regression analyses revealed no significant relationships between significant sleep parameters (derived from either PSG data or self-reports) and memory-forgetting indices in any group. Therefore, comparisons between regression slopes were not performed.

Sleep Misperception in Healthy Aging and MCI

Figure 2 illustrates group differences between PSG-objective and subjective estimation of the SOL and sleep duration. We found that only the SOL was significantly overestimated (F5,48 = 9.3, P < 0.004), showing a trend toward significance for the interaction effect (F1,46 = 3.5, P < 0.07). In agreement with this trend, post hoc analyses revealed that patients with MCI overestimated the SOL (P < 10^-4; self-reports = 34.3 ± 4.8; PSG = 14.7 ± 1.5) compared with HE subjects (self-reports = 19.2 ± 4.7; PSG = 11.3 ± 1.5). The presence of the ε4 allele in patients with MCI did not influence sleep perception.

DISCUSSION

The current study provides compelling evidence of objective and subjective sleep disturbances in patients with MCI, suggesting that sleep problems precede in years the clinical onset of AD, and therefore adding support to a positive feedback loop between impaired sleep and Aβ levels previously reported in animal models of AD.24 Our results further indicated...
a poor correspondence between objective sleep physiology and subjective estimates of sleep in this preclinical population. In particular, patients with MCI were unable to establish associations between SWS fragmentation and self-reports related to sleep quality, and they also exhibited significant discrepancies in the estimation of SOL when compared with HE subjects.

Sleep Disturbances in MCI: From Objective Physiology to Self-Reported Measures

Evidence suggesting that aging-related cognitive decline could be exacerbated by a loss of sleep integrity has fed the hypothesis that sleep disturbances in older adults might anticipate AD, which has been recently supported by studies using mouse models of β-amyloidosis. These studies showed that sleep disruptions appeared after plaque formation, and reversed after elimination of Aβ deposits. Given that Aβ aggregation become evident years before the clinical onset of AD, examining sleep disturbances during preclinical stages of AD might have important implications for early diagnosis and disease progression.

The belief that MCI status is the preclinical stage of AD has received strong support from neuropathological, biochemical, neuroimaging, and neurophysiological findings. Although overnight PSG studies are rare in patients with MCI, previous research using subjective measures concur that sleep problems are more frequent in patients with MCI than in HE subjects. The current study corroborates these findings by using five sleep questions focused on aspects of sleep related breathing and/ or sleep movement disorders, underlies sleep onset misperception in our MCI population. Although we did not objectively exclude the presence of sleep apnea and/or periodic limb movements in our sample, subjective levels of daytime sleepiness did not differ between groups, and ESS scores were in all cases below the threshold for suspecting sleep disorders associated with excessive daytime sleepiness. Furthermore, neither study participants nor their bed partners reported symptoms associated with these sleep disorders. We therefore believe that sleep onset misperception is intensified by memory deficits in patients with MCI, providing novel insight into the interaction between early neurodegeneration, and sleep perception in preclinical stages of AD.

Possession of one or two copies of the ε4 allele in the ApoE gene has been suggested as the major genetic risk factor for developing AD in patients with MCI. Whereas REM deficits in patients with MCI are aggravated by the presence of the ApoE ε4 genotype, this condition was neither associated with higher prevalence of self-reported sleep disturbances nor predicted significant relationships between objective and subjective sleep alterations. Recent studies have found that human ApoE4-targeted replacement mice, but not wild-type control mice, showed significant reduction of SWS and REM sleep during acute exposure to intermittent hypoxia and sleep fragmentation. Building on these findings, we suggest that physiological but not subjective sleep is more vulnerable to the presence of the ε4 allele in patients with MCI. However, further longitudinal studies are needed to establish whether MCI ApoE ε4 carriers showing altered PSG sleep patterns convert faster to AD than those only showing subjective sleep complaints.
This study has several potential sources of bias that should be noted. First, we compared PSG sleep data recorded in 1 night with subjective sleep quality over the past few months to establish relationships between objective and subjective sleep in HE subjects and patients with MCI. This approach implicitly assumes that PSG data obtained from one single night is representative of a typical night in the past few months. A more reliable approach would have been to correlate overnight PSG recordings with self-reports of sleep referring to that particular night. Second, PSG sleep studies were performed without previous adaptation of participants to the sleep laboratory. As a consequence, our results may be affected by the first-night effect (i.e., differences observed on the first PSG sleep recording in comparison with consecutive ones), which has previously been demonstrated to affect older patients more than younger ones. This hypothesis is, however, less plausible because the effect of the first-night effects on sleep structure would be expected to be similar in both HE subjects and patients with MCI.

**Basal Forebrain: Where AD Meets Sleep and Cognition**

Different regions of basal forebrain (BF) are involved in nonrapid eye movement (NREM) sleep regulation, as revealed by lesion and stimulation studies. In addition, several studies have shown that NREM sleep is significantly reduced in patients with AD. Convergent evidence further suggested that BF cholinergic neurons are selectively vulnerable to AD neurodegeneration, adding support to the hypothesis that cholinergic dysfunctions are partially responsible for the cognitive deficits observed in patients with AD. We have recently extended this hypothesis to preclinical stages of AD, showing that patients with MCI exhibited significant volume reductions of the nucleus basalis of Meynert that in turn correlated with impaired cognition in this preclinical population. Therefore, damage of BF nuclei together with altered SWS might exacerbate cognitive dysfunctions in patients with MCI.

Convergent evidence also supports relationships between impaired sleep and lesions of BF nuclei in patients with MCI. First, BF regions involved in SWS regulation are damaged in patients with MCI. Second, Aβ plaques appear years before the clinical onset of AD, likely triggering a positive feedback loop between Aβ concentrations and sleep-wake irregularities during preclinical AD stages. In line with the second hypothesis, increased disruption of SWS might lead to impaired cognitive integrity due to higher Aβ concentrations, which in turn might contribute to sleep misperception observed in patients with MCI. Recent evidence has shown that sleep disturbances increase proinflammatory cytokine levels and further induce microglia activation in the mouse hippocampus, leading to deficits in hippocampal-dependent learning and memory consolidation. Collectively, these findings suggest that SWS disruptions reported in patients with MCI could both activate the amyloid cascade and induce neuroinflammation in the hippocampus. Both complementary conditions pave the way to AD progression.

No significant associations were found between SWS fragmentation and memory performance/forgetting rate, neither in HE subjects nor in patients with MCI. However, this lack of significance does not allow us to fully discard associations between sleep integrity and memory performance in prodromal stages of AD. Future investigations should include memory indices more sensitive to AD neuropathology. For instance, patients with MCI have more difficulties in remembering relations among items or between an item and its context (associative memory) rather than individual items. In support of this hypothesis, we recently found in patients with MCI significant correlations between gray matter volume of the entorhinal cortex and associative memory deficits, but not with immediate or delayed recall. Therefore, indices of associative memory might be more appropriate to investigating potential relationships between memory performance and sleep integrity in MCI patients.

Only one study has previously evaluated potential relationships between objective and subjective measures of sleep in early to moderate stages of AD, although objective sleep parameters were obtained with actigraphic recordings. Authors found significant discrepancies between objective and subjective sleep in patients with AD, but not in control subjects. Our results confirm these findings and allow us to extend sleep misperception to years before the clinical onset of AD, which might result from complex interactions between sleep disruptions and high levels of Aβ. Much more research is needed to fully understand relationships between sleep disturbances and Aβ levels during the continuum healthy aging to severe AD, and to establish whether improving sleep in preclinical stages of AD is a beneficial strategy in slowing the progression of this neurodegenerative condition.

**CONCLUSIONS**

Sleep complaints are commonly underdiagnosed in the geriatric population although they constitute a significant source of concern in patients with dementia. The current study confirms that sleep disturbances in patients with MCI can be determined based on the results of both overnight PSG recordings and self-reports. Our results further revealed that patients with MCI not only are unable to establish coherent relationships between objective and subjective sleep but they also have significant difficulties in correctly estimating the SOL, which might result from memory deficits intrinsic to this preclinical condition. Taken together, these results add support to reciprocal relationships between impaired sleep and Aβ levels, suggesting that this positive feedback loop begins years before the clinical onset of AD. Results of the current study have also important implications for early diagnosis of AD, and might aid in the development of novel therapeutic strategies devoted to improve sleep in elderly patients with impaired cognition.

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**DISCLOSURE STATEMENT**

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