A great interest has emerged in the last 20 years in finding biological markers of different diseases and psychiatric disorders. The series of studies we undertook was an attempt to find such a marker for Alzheimer's Disease (AD), through the analysis of sleep architecture and sleep microstructure variables and quantitative EEG analyses. One of the long-term goals was to be able to better evaluate the effects of pharmacological treatments of AD.

Sleep variables

One aspect of our research is to study sleep architecture and microstructure in mild to moderate AD (Montplaisir et al., 1995b). Our main finding is that REM sleep percent was reduced in AD patients compared to controls and this, as a result of a decrease in mean REM episodes duration. Other REM sleep variables, such as REM atonia, eye movement density, phasic EMG, number of REM episodes and REM latency, were all unchanged for the total REM sleep periods. In other words, variables pertaining to the initiation of REM sleep and to its characteristic features were unaffected in mild AD. This is probably because these variables are under the control of the mesopontine cholinergic populations, structures which are spared in mild AD. The lower REM sleep percentage, however, could be due to degeneration of the nucleus basalis of Meynert (NB). This nucleus normally exerts an inhibitory influence on the nucleus reticularis of the thalamus (Buzsaki et al., 1988), the rhythm generator responsible for NREM sleep. If the NB degenerates to the point of being unable to fully activate the cortex during wakefulness and REM sleep, its inhibitory influence on the nucleus reticularis might be weakened, leading to the curtailment of REM sleep episodes. This model suggests that a reduced activity in the NB, as during immobility or following a lesion, would result in an increase of slow delta waves. A similar observation can be expected in case of degeneration of the NB as in AD. However, percentage of NREM sleep did not vary significantly in our studies of AD patients as compared to previous studies (Prinz et al., 1982; Vitiello et al., 1990), probably because our group of patients were in mild stages of the disease. In contrast, the analysis of sleep microstructure revealed a decreased number of sleep spindles and K-complexes in AD patients compared to normal elderly subjects.

Quantitative EEG

We reasoned that the power of the EEG in diagnosing AD might be enhanced if it were assessed using REM sleep instead of wakefulness. The rationale was that the cholinergic basal forebrain, which is implicated in cortical activation and which
degenerates early in AD (Whitehouse et al., 1982), is likely to be more crucial for EEG activation of REM sleep than of wakefulness. Indeed, the EEG activation achieved in wakefulness is the result of many convergent neuronal and neurotransmitter systems, many of which (including norepinephrine and histamine) are silent during REM sleep (Hobson et al., 1975; Sakai et al., 1990). This leaves mainly the cholinergic nucleus basalis (Buzsaki et al., 1988) and glutamatergic thalamo-cortical cells (Steriade and McCarley, 1990) to ensure EEG activation during the latter state. Thus, REM sleep EEG might be more likely to serve as a biological marker of AD than the awake EEG.

This is indeed what we have found. Slowing is more prominent in the REM sleep EEG than in the waking EEG of AD patients. When only the temporal regions, the cortical areas most and first affected in AD, are considered, the REM sleep EEG correctly classified 100% of controls and AD patients at an early stage of the disease (Petit et al., 1992). This first study was, however, conducted on a modest number of AD patients (n=8) and controls (n=8). The observed EEG slowing was the result of a change in all four classical frequency bands: an increase in percent power in delta and theta bands and a decrease in the percent power in alpha and beta bands. Furthermore, there was a distinct topographical pattern of REM sleep EEG slowing in AD patients which is in agreement with findings from neuroradiological and neuropathological studies and which was not observed for the waking EEG (Petit et al., 1993a). In REM sleep, the EEG slowing was indeed the most pronounced in the temporal regions, next most pronounced in the parieto-occipital, then the frontal regions, whereas the controls showed the same cortical activity values for all three regions studied. The REM sleep EEG was indeed correlated with a global assessment of cognitive functioning (the Mini-Mental State) and with interhemispheric asymmetry of regional cerebral blood flow by Single Photon Emission Computerized Tomography (Montplaisir et al., 1996).

The premortem diagnosis of probable or possible Alzheimer Disease is obtained exclusively from clinical examination. Without neuropathological confirmations, there are ambiguities concerning the reliability of the diagnosis, especially in mild cases, where the evolution of the clinical manifestations of AD are highly variable. As mentioned previously, the discriminative power of EEG between AD patients and control subjects, especially in REM sleep, demonstrated a high sensitivity and specificity in our earlier studies. Since diagnosing AD is troublesome in the first stages of the disease, the spectral EEG analysis yields additional objective and repeatable data for an early diagnosis of this heterogenous pathology.

To better pinpoint brain regions presenting significant cortical slowing, significance probability mapping was used in conjunction with a more complete EEG recording (Hassainia et al., 1994). It was confirmed that for REM sleep, EEG slowing was greater in the temporo-parietal and frontal regions, whereas for wakefulness, EEG slowing was greater for the frontal pole. Moreover, in order to reevaluate the discriminative power of our measure, a new and larger group of mild to moderate AD patients (n=27) and of controls (n=25) were studied with a 16-channel 10-20 recording montage (Hassainia et al., 1997). Using the ratio of slow over fast frequencies from the temporal regions, a correct classification of 90.4% of subjects (sensitivity: 81.5%, specificity: 100%) was obtained for the REM sleep EEG. The best discrimination rate for the waking EEG, obtained from the frontal regions, was only 80.8% (sensitivity: 66.7%, specificity: 96%). The discrimination rate obtained with the REM sleep EEG is the best marker of AD so far using a single measure. Tonic REM sleep also has the advantage over wakefulness of being a fairly homogenous state; it does not pose problems concerning variations in the patient’s level of vigilance.

Role of brainstem and forebrain cholinergic populations

The implication of brainstem and forebrain cholinergic neurons in sleep-wake cycle regulation is well known. Knowledge of the role of these structures is based mostly on pharmacological, neuroanatomical, biochemical and electrophysiological studies in animals. The interest for the cholinergic mechanisms of REM sleep control probably began in the early 1960’s with Jouvet and his first pontine lesion experiment in the cat (Jouvet, 1962). Later, he showed (Jouvet, 1969) that, in a pontine cat, REM sleep could be enhanced by the anticholinesterase eserine and suppressed by the muscarinic antagonist atropine. Moreover, injection of hemicholinium which blocks uptake of choline, the biosynthetic precursor of ACh, decreases REM sleep in cats (Domino and Stawiski, 1970; Hazra, 1970).

The understanding of human REM sleep is more obscure and derives from pharmacological studies using receptor blocking agents, cholinesterase inhibitors and muscarinic antagonists. Indeed, consistent with animal studies, scopolamine and atropine decrease or suppress REM sleep in normal subjects without affecting total sleep time (Sagales et al., 1975; Toyoda et al., 1966). In contrast, cholinomimetic agents like physostigmine or arecoline are known to increase REM sleep by inducing REM sleep episodes with no influence on their duration (Sitaram and Gillin, 1980).

We have studied further the differential role of brainstem and forebrain cholinergic populations by conducting a similar study in PSP patients (Montplaisir et al., 1997). In this neurodegenerative disease, one of the structures most affected (along with substantia nigra and basal ganglia) is the cholinergic pedunculopontine nucleus (PPT) of the brainstem (Jellinger, 1988). Since PPT is centrally involved in the control of REM sleep initiation and its characteristic features, it was expected that REM sleep variables would be altered in patients with PSP. Although the nucleus basalis of Meynert is also affected to a certain degree by cell loss, the remaining intact neurons do not demonstrate a significant reduction in nuclear volume, suggesting normal neurotransmitter production (Mann, 1982). Thus, a significant degree of EEG slowing in REM sleep was not expected for PSP patients.

We found that the percentage of REM sleep was lower in PSP patients than in controls. This was due to both a lower mean REM episode duration and a tendency to have fewer REM sleep episodes. REM density was also significantly reduced in these patients. Although mean latency to the first
REM episode was not significantly different from that of controls, it showed much more variability in PSP patients.

In awake PSP patients, a slowing of the EEG (as determined by a spectral ratio) was found for the 6 frontal leads, C4, P4 and T4 compared to control subjects. For the REM sleep EEG, there were no significant between-group differences in the spectral ratio for any of the 16 leads studied. The frontal EEG slowing during wakefulness is consistent with the results of numerous neuropsychological studies which show deficits to be related to frontal lobe functions (Dubois et al., 1988; Maher et al., 1985). The fact that no EEG slowing was found in REM sleep suggests that the slowing observed for wakefulness was not likely due to a cholinergic deficit. This is consistent with findings that normal neocortical and hippocampal choline acetyltransferase activity was found in some PSP patients (Kish et al., 1985). Dopamine levels are, however, severely reduced in the caudate, putamen and substantia nigra in PSP patients (Kish et al., 1985). A frontal deafferentation from the striato-pallidal complex is thought to be responsible for the impairment since there are extensive fiber connections between these nuclei and the prefrontal region. Indeed, the positive correlations between degree of impairment on frontal tasks and EEG slowing observed in our PSP patients suggest that both impairments could be the result of a dopaminergic deficiency.

Evaluation of pharmacological treatments of AD

The final aspect of our work was the evaluation of pharmacological treatments acting upon the cholinergic system in mild to moderate AD patients. Our first attempt was with tetrahydroamino-acridine (THA ou tacrine), an acetylcholinesterase inhibitor (Petit et al., 1993b). THA did not significantly alter any EEG variable. Compared with the placebo condition, THA also had no significant effect on mean EEG spectral power for either wakefulness or REM sleep. However, a subgroup of AD patients seemed to respond to the drug, as both their Mini-Mental State scores and quantitative EEG in REM sleep over time.

Clinical trials of experimental drugs for AD are often based on a six-month evaluation and are aimed at stopping the progression of the disease rather than reversing the process. However, there are not a lot of studies investigating what happens to EEG spectral power in a six-month period in mild AD. Previous studies reported that slowing of the awake EEG progressed with time (Coben et al., 1983; Fenton, 1986; Helkala et al., 1991; Penttilä et al., 1985; Sloan and Fenton, 1993) but no study has ever looked at the changes of quantitative EEG in REM sleep over time.

**SHORT REPORT**

**Methods**

We present here preliminary data from a new study aimed at exploring the modifications of the EEG spectral composition in patients with mild to moderate AD in wakefulness and in REM sleep over time.

Eight AD patients (3 men and 5 women; mean age 68.4 years) were reassessed six months following the first investigation. All patients met the NINCDS-ADRDA criteria of probable Alzheimer's disease (McKhann et al., 1984). Patients were at mild to moderate stages of AD, i.e., stages 3 and 4 of Reisberg’s scale (Reisberg et al., 1982). None of the subjects had a history of drug or alcohol abuse and all had been free of psychotropic medication for at least two weeks prior to the recordings.

Subjects were recorded in the sleep laboratory for one baseline night and one night six months later. Sixteen electrodes were placed according to the international 10-20 system, using a referential montage. A 16-channel Grass polygraph (amplifier gain 7.5µV/mm, bandpass 0.3-100 Hz) was used to amplify the signals and record them on paper. The signals were also relayed to a computer where they were digitized at a sampling rate of 128 Hz and filtered with a digital filter having a cutoff frequency at 64 Hz. Amplitude spectral analyses by fast Fourier transform were performed on artifact-free sections (96 s in total) of awake and of tonic REM sleep.

**Table 1**

Mean Values for Absolute Activity in Different Frequency Bands for 8 Alzheimer Patients

<table>
<thead>
<tr>
<th>Band</th>
<th>Stage</th>
<th>Baseline Mean ± SD</th>
<th>After Six Months Mean ± SD</th>
<th>Wilcoxon p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta</td>
<td>Wake</td>
<td>38.71 ± 14.71</td>
<td>37.71 ± 11.14</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>REM</td>
<td>48.19 ± 12.50</td>
<td>48.92 ± 18.68</td>
<td>0.67</td>
</tr>
<tr>
<td>Theta</td>
<td>Wake</td>
<td>56.48 ± 25.23</td>
<td>53.98 ± 27.43</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>REM</td>
<td>40.24 ± 7.31</td>
<td>42.40 ± 17.40</td>
<td>0.89</td>
</tr>
<tr>
<td>Alpha</td>
<td>Wake</td>
<td>56.63 ± 31.95</td>
<td>49.39 ± 28.92</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td>REM</td>
<td>26.26 ± 6.83</td>
<td>27.78 ± 10.39</td>
<td>0.48</td>
</tr>
<tr>
<td>Beta</td>
<td>Wake</td>
<td>24.89 ± 9.15</td>
<td>23.32 ± 10.09</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>REM</td>
<td>13.84 ± 3.83</td>
<td>13.47 ± 3.83</td>
<td>0.40</td>
</tr>
</tbody>
</table>
EEGs. The awake EEG was recorded while patients were lying on the bed with eyes closed on the morning following the night recording. The samples of REM sleep were visually selected from the first, middle and last REM sleep periods. Four frequency bands were defined as: delta (0.75 to 3.75 Hz), theta (4 to 7.75 Hz), alpha (8 to 12.75 Hz) and beta (13 to 20.25 Hz).

**Results**

The expected increase in low frequency bands was not observed in this group of AD patients six months after the first recording. In fact, changes in delta and theta bands were slight and non-significant during both wakefulness and REM sleep (Wilcoxon, p>0.21; Table 1). A marked reduction in absolute alpha activity was the only factor contributing to EEG slowing. This decrease in alpha activity was significant, specifically during wakefulness, for all five cortical regions investigated: frontal, central, occipital, parietal and temporal (Wilcoxon p<0.04; Table 2). The decrease in alpha activity during wakefulness was found in all eight AD patients. No significant changes were found during REM sleep for the alpha band in any cortical region.

The decrease in the alpha activity during wakefulness seems to parallel the evolution of AD, this modification following in time the increase in slow frequency bands. Coben et al. (1985) reported indeed that a low alpha activity is a phenomenon observed in more advanced stages of AD patients. The important reduction in alpha power observed in eight AD patients after six months indicates that these changes could be related to the rate of progression of the disease, at least for a brief period of time.

Many studies reported that the decline in the power of high frequencies was correlated with advancing stages of dementia and with a decline in neuropsychological performance (Brenner et al., 1986; Duffy et al., 1984; Penttila et al., 1985; Streletz et al., 1990). A low absolute alpha power has been reported to parallel poor performance on the MMSE test (Kuskowski et al., 1993), a global measure of cognitive decline in AD patients. Our observations are in agreement with this view. This biological marker of AD might eventually have important implications for adequate follow-up and care of AD patients. It may also allow prospective studies in patients with a positive neuropsychological diagnosis and a normal EEG on visual inspection; a mild EEG slowing might be detected at one point. It would also be interesting to investigate if EEG abnormalities can be observed before cognitive decline in individuals who would eventually develop AD.

**Discussion**

In conclusion, the study of sleep variables in specific neurodegenerative diseases can help to identify the role of different sleep-generating structures in humans. Quantitative EEG measures, especially those derived from REM sleep, can be used not only as a diagnostic tool but also to evaluate patients’ biological responses to cholinergic treatment by a reactivation or a stabilization of EEG parameters.

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