Memorization test and resting state EEG components in mild and subjective cognitive impairment

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Abstract. Background: Mild (MCI) and Subjective Cognitive Impairment (SCI) are conditions at risk of developing Alzheimer's disease (AD). Differential between normal aging at early stages can be really challenging; available biomarkers need to be combined and can be quite invasive and expensive. **Objective:** The aim of this pilot study is to examine possible EEG alterations in MCI and SCI compared to controls, analyzing if a cognitive task could highlight early AD hallmarks. **Method:** We recruited 11 MCI, 8 SCI and 7 healthy subjects as controls (CS), all matched for age and education. Neuropsychological assessment and EEG recording, at resting state and during a mental memory task, were performed. Classical spectral measures and nonlinear parameters were used to characterize EEGs. **Results:** During cognitive task, α-band power reduction was found predominantly in frontal regions in SCI and CS, diffused to all regions in MCI; moreover, decreased EEG complexity was found in SCI compared to controls. The α-band power attenuation restricted to frontal regions in SCI during a free recall task (involving frontal areas), suggests that MCI patients compensate for encoding deficit by activating different brain networks to perform the same task. Furthermore, EEG complexity reduction - that has been found already in SCI - could be a possible early hallmark of AD. **Conclusion:** This study draws attention on the importance of nonlinear approach in EEG analysis and the potential role of cognitive task in highlighting EEG alterations at very early stages of cognitive impairment; EEG could therefore have a practical impact on dementia diagnosis.

Keywords: Mild Cognitive Impairment (MCI), Subjective Cognitive Impairment (SCI), early Alzheimer's disease, memorization task, nonlinear EEG analysis, EEG signal complexity

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterized by a gradual progressive decline in memory and other cognitive domains that affects functioning of daily activities. It has been suggested that AD begins years, probably even decades, before the appearance of the first cognitive symptom [1], but the distinction between normal ageing and AD at a very early stage is still a challenge.

There are currently two recognized pre-dementia stages: Subjective Cognitive Impairment (SCI) [2] and Mild Cognitive Impairment (MCI) [3], [4], that differ according to the absence or presence of impairment on cognitive testing, respectively, without impact on patient's functional status. Both are considered conditions at high risk for developing AD. Therefore, detection of AD hallmarks at an early stage, before major brain damage and functional impairment have occurred, is an important goal in the management of these patients; this is even more relevant considering the growing research of drugs specifically targeting AD molecular pathways.

Clinical diagnosis of AD typically relies on patient's history and neuropsychological tests, supported by evidence of Aβ protein deposition and downstream neurodegeneration, achieved by neuroimaging (Magnetic Resonance Imaging, fluorodeoxyglucose positron emission tomography (18F-FDG PET), amyloid PET [5], [6]) and spinal fluid biomarkers analysis.

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However, these biomarkers have different sensitivity and specificity in AD early diagnosis, and some of these procedures are quite invasive and expensive, therefore hardly feasible in routine clinical setting.

Amyloid plaques and neurofibrillary tangles pathogenic accumulation has been associated with local synaptic disruptions, suggesting that AD is a *disconnectivity* disease [7], [8]. Progressive impairment of use-dependent synaptic plasticity and synaptic connectivity between neurons are considered a neurophysiological hallmark of brain aging, confirmed by their association with the degree of dementia [9]. A useful instrument to disclose intra-brain associations, through direct recording of brain's electrical activity, is Electroencephalogram (EEG) [10]. This tool is very useful for screening purposes thanks to its wide availability, relatively low cost, short duration and non-invasivity. According to AD disconnectivity theory, EEG could potentially be a promising tool for early detection of cognitive impairment [11].

Several studies have found common electroencephalographic alterations in AD patients: increase of delta and theta bands power and decrease of alpha and beta bands power [12]–[16]. Studies based on complexity analysis, such as the entropy method, also showed that EEG signals in AD patients had reduced complexity than controls and that resting state synchrony among brain regions may be reduced [17].

Some studies have tried to investigate EEG alterations in MCI due to AD [15], [18]–[24] and few studies considered patients with Subjective Cognitive Impairment (SCI) [25], [26]. Most of those studies were based on resting EEG recordings, not during cognitive stimulation, while spectral analysis of EEGs recorded during a memorization task was unable to distinguish between MCI patients and control group [27].

Experimental results on MCI subjects revealed intermediate posterior alpha rhythms between elderly control and AD subjects, increased theta and delta power in temporal and occipital regions and decreased beta power in temporal and occipital regions compared to control subjects [11]. Conversely, EEG changes in SCI subjects are more debated, but quantitative evaluation of EEG at resting state showed abnormal delta, theta and alpha sources compared to normal elderly [25].

The aim of this study was to examine possible early alterations in brain oscillatory activity in MCI and SCI groups compared to control, investigated in resting state and during cognitive task. More in detail, we wanted to check if a cognitive stimulation could be able to highlight early AD hallmarks on EEG. We therefore applied different EEG methods as linear spectral measures and nonlinear parameters inspired by chaos theory, in order to be more sensitive in detecting early pathological markers of cognitive decline. If the linear approach is a well-established tool for EEG analysis, the nonlinear one has been recently introduced, proving to be valuable for the characterization of many physiological and pathological conditions [28]–[31].

2. MATERIALS AND METHODS

2.1. Study population

Patients were recruited at the Neurological Unit - Memory Centre - of the University Hospital "Ospedali Riuniti" in Trieste. The study included 26 subjects, aged between 65 and 85 years: 11 MCI, 8 SCI and 7 control subjects (CS). The MCI group was composed of 8 females and 3 males, mean age was 76.8 years (range 67-85), mean education 8.8 years (range 5-13), average MMSE score 27 (range 24-28) and for one patient MOCA (score 24/30) was used. The SCI group was composed of 5 females and 3 males, mean age 74.6 years (range 65-82), mean education 11.5 (range 8-18), average MMSE score 28.6 (range 26-30) and for three patients MOCA (score range 26-27) was used. Finally, the control group was composed of 3 females and 4 males matched with patients for age and education, mean age 74.3 years (range 67-84), mean education 8 years (range 5-13), average MMSE score 28.5 (range 25.4-30). Demographic variables, global cognitive function and depression assessment are reported in table 1.

MCI diagnosis was based on the diagnostic criteria published in 2011 by the National Institute on Aging and Alzheimer's Association [4]. Inclusion criteria for the SCI group were presence of subjective memory complains since less than 5 years and absence of objective cognitive impairment on neuropsychological assessment. Inclusion criteria for the Control group were absence of objective/subjective cognitive impairment and of any other neurological or psychiatric disease. For all groups exclusion criteria were other possible causes of dementia (vascular encephalopathy, other degenerative diseases, etc.), severe traumatic brain injury, marked depression (Hamilton Rating Scale For Depression score >7) and psychotropic drug therapy.

All subjects underwent general physical examination, neurological examination and neuropsychological evaluation. MCI and SCI patients underwent laboratory testing (thyroid, liver and kidney function, B12, folate, electrolytes and blood cells count) and neuroimaging scan (Computed Tomography (CT) or Magnetic Resonance Imaging (MRI)), in order to rule out reversible causes of cognitive impairment.

2.2. Neuropsychological assessment

Standardized neuropsychological tests were administered as clinical diagnostic tool, allowing the distinction between MCI, SCI and CS. Memory, attention, language, praxic-constructive and visual long term memory functions were assessed.

Global cognitive impairment was evaluated using Mini−Mental State Examination (MMSE) [32], corrected for age and education [33], a screening test for mental deterioration, assessing the following five areas: orientation to time and place, immediate recall and short-term verbal memory, attention and calculation, delayed recall, language and constructional ability. In some patients, Montreal Cognitive Assessment (MOCA) [34] was used; this is a more comprehensive cognitive screening battery that covers most cognitive domains (visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall and orientation) and is more sensitive to detect Mild Cognitive Impairment, irrespective of etiology.

Depressive symptoms were measured using the 17-item Hamilton Depression Scale [35].

Anterograde long-term memory was evaluated using Prose Memory Test [36], [37] , assessing the ability in memorizing a short story and recalling it immediately (Immediate Recall) and 15 minutes later (Delayed Recall). Attentive functions were evaluated using Attentive Matrices Test [37] for visual selective attention and Trail Making Test (TMT) Parts A and B [38], [39] for psychomotor speed, visuospatial research, selective and divided attention. Rey complex figure [40]–[42] was used to evaluate praxic-constructive (Immediate Copy) and visual long-term memory abilities (Delayed Recall after 15 minutes). Phonemic and Semantic Verbal Fluency Tests [36], [37] were used to evaluate the access to mental lexicon and the ability to select correct words without repetition (which requires executive functions and mental control).

Table 1. Demographic variables, global cognitive function and depression assessment.

Group	*Case*	*Sex*	*Age* (years)	*Education* (years)	*Duration of disease* (months)	*MMSE* (/30)	*MOCA* (/30)	*HRSD*
MCI	CE	F	76	5	35	28		0
	DPE	M	79	5	8	26		7
	DS	M	73	13	9	24.7		0
	TR	F	67	7	47		24	3
	MC	F	85	7	57	27.4		9
	UM	F	76	13	24	24		0
	LA	F	72	10	33	27.4		6
	FA	F	75	6	86	28		6
	ML	F	80	13	4	26.7		0
	UM	M	81	11	60	26.7		0
	RDA	F	80	7	12	25.4		0
SCI	GB	F	72	14	34	30		3
	SO	F	71	10	8	30		7
	PS	F	65	8	50		26	0
	MA	M	73	13	42	30		0
	RE	F	82	8	12	27.7		2
	CD	M	82	18	60		27	0
	RG	M	75	13	24		26	5
	CR	F	77	8	36	27.7		1
CS	GV	F	67	8	/	30		3
	KR	M	72	8	/	30		4
	RM	F	75	7	/	28.7		4
	DAF	M	73	13	/	26.7		0
	BG	M	76	5	/	26.4		0
	SR	F	73	8	/	27.4		0
	MG	M	84	7	/	30		0

Note: Subjects are reported by their initials; F = female. M = male; MMSE = Mini Mental State Examination [Folstein et al., 1975]; MOCA = Montreal Cognitive Assessment [Nasreddine et al., 2005]; HRDS = Hamilton Rating Scale For Depression [Hamilton, 1960].

2.3. Experimental design

Two EEG recordings, performed from 19 electrodes positioned according to the International 10-20 System (sampling frequency 1024Hz), were acquired from each subject before and during a memory task. At first, a 5-minute EEG recording in resting state (EEG 1) with closed eyes was recorded. After this a memory task was performed: each subject was asked to listen to an oral presentation of the Rey's 15 word list [43], [44] and then to immediately repeat the words they were able to recall, without time constraint (Rey's 15-word Immediate Recall). This task was repeated for five times as a learning period, followed by 15 minutes of distractor cognitive tasks, not involving memory and/or learning (e.g. attentive test). Afterwards, a second two minutes EEG recording was performed while the subject was asked to close his eyes and mentally recall the words of the Rey's list presented before (EEG 2). Finally, the subject was asked to repeat the words he was able to recall, with no time constraint (Rey's 15-word Delayed Recall).

The choice of this task was based on two reasons: 1) it is a semantic encoding and recall task with high sensitivity and specificity in detecting cognitive impairment due to AD, even at early stages [45]–[47]; 2) a mental recall task can be performed with eyes closed and motionless, minimizing EEG artifacts.

The study was conducted according to the Declaration of Helsinki and written informed consent for the use of the EEG data was obtained from the patients.

2.4. EEG features extraction

The recorded EEG data were filtered with a second order band-pass Butterworth filter with cutoff frequencies of 0.5 and 60 Hz. For each testing condition, data with muscular, ocular and other types of artifacts were manually discarded and 60 seconds of stationary EEG signal were selected (see an example in

Fig.1). Only these segments were accepted for further analysis.

The analysis in the frequency domain was performed using Welch's periodogram method. Recordings were segmented into tracts of 10 seconds each, windowed with a Hanning window, with 50% overlap. The relative powers of the spectral components in the typical spectral bands [48] delta (δ: 0.5-4 Hz), theta (θ: 4-8 Hz), alpha1 (α1: 8-10.5 Hz), alpha2 (α2: 10.5-13 Hz), beta1 (β1: 13-20 Hz), beta2 (β2: 20-30 Hz) and gamma (γ: 30-60 Hz) were computed by dividing the absolute power in each band by the total power. As additional spectral features, the absolute power in the whole band (total power in the 0.5-60 Hz band) and the individual alpha frequency peak (IAF peak), defined as the frequency associated with the strongest EEG power at the extended alpha range (7-14 Hz), were also calculated [49].

In order to analyze the nonlinear self-similarity behavior of the considered EEG time series in tract as short as those examined with linear analysis (i.e. 10s) we evaluated both the power-law beta exponent (β exponent) and the fractal dimension (FD) of the signal. The power versus frequency relationship was investigated in a log-log plot and the power-law beta exponent was calculated as the slope of the regression line fitting the power spectral density [50], as shown in figure 1. On the other hand, FD values were calculated directly from EEG signals by means of the Higuchi's algorithm [51].

All the proposed parameters were separately calculated for each electrode. Successively, in order to group information coming from a specific brain region, averaged measures were computed and the 19 channels were grouped into scalp regions based on their locations: (LF) left frontal (Fp1, F3, F7) , (RF) right frontal (Fp2, F4, F8), (LT) left temporal (T3, T5), (RT) right temporal (T4,T6), (C) central (C3, C4, Cz), (P) parietal (P3, P4) and (O) occipital (O1, O2) areas.

This EEG analysis approach has been widely explained in an our conference paper [52], in which some preliminary results of the present work were also presented.

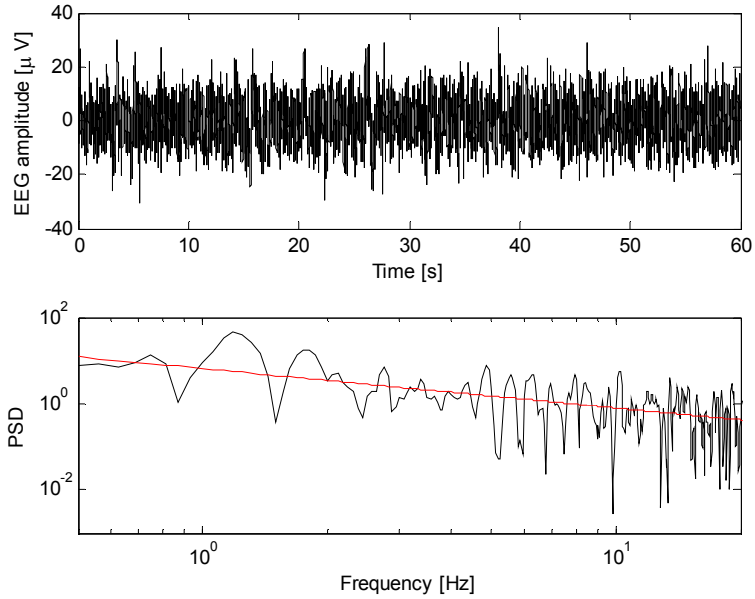

Figure 1. Example of EEG signal on the left and its 1/f-like spectrum on the right (beta exponent=0.91).

2.5. Statistical Analysis

Due to the low samples size, the nonparametric Kruskal-Wallis test was used to compare the three groups (CS, MCI and SCI) and the Wilcoxon rank sum test was used to compare each pair of groups, followed by Bonferroni's correction due to multiple testing. A Wilcoxon paired two-sided signed rank test was performed to compare, for each group, data concerning memorization task and resting state condition. Differences were considered significant for a p-value < 0.05. Medians with 25th and 75th percentile were calculated for each parameter and each group of subjects.

3. RESULTS

3.1. Memory task results

Scores obtained on the Memory task performance (adjusted for age and education) are reported in table 2. At the Rey's 15-word Immediate Recall MCI average equivalent score (ES) is 1.54 (range 0-3), SCI average ES is 2.87 (range 0-4) and CS average ES is 3.71 (range 3-4). At the Rey's 15-word Delayed Recall MCI average ES is 1.18 (range 0-2), SCI average ES is 3.37 (range 1-4) and CS average ES is 3.57 (range 2-4).

Table 2 Memory task performance

Group	*Case*	***Rey's 15-word Immediate Recall***		***Rey's 15-word Delayed Recall***	
		score	*E score*	*score*	*E score*
MCI	CE	23	0	6.1	2
	DPE	34	2	3.1	0
	DS	27.1	0	0	0
	TR	38.1	3	3.8	1
	MC	33.2	2	0	0
	UM	29.2	1	0	0
	LA	31.9	1	4.9	1
	FA	40	3	6.1	2
	ML	31.4	1	5.6	1
	UM	31.1	1	0	0
	RDA	39.2	3	5.8	2
SCI	GB	34.1	2	9.2	4
	SO	51.9	4	8.9	4
	PS	40.4	3	9.3	4
	MA	33.1	2	7.2	3
	RE	47.1	4	9.3	4
	CD	27.6	0	5.2	1
	RG	41.2	3	7.9	3
	CR	44.9	4	11.6	4
CS	GV	41.3	3	12.7	4
	KR	43.9	4	9.9	4
	RM	43	4	10.1	4
	DAF	45.1	4	11.75	2
	BG	37	3	10.1	3
	SR	44.9	4	7.9	3
	MG	44.2	4	8.8	4

Note: Subjects are reported by their initials;
E score = equivalent score

3.2. Comparison of EEG features among groups

Table 3 presents the median values (with 25th and 75th percentiles) of the parameters that showed significant differences ($p < 0.05$) among the groups at the Kruskal-Wallis test, calculated from single channel and from brain region, respectively.

The analysis made on single channel measures showed that during resting state differences were significant only between MCI and SCI groups and mainly concerned frontal and central regions, with only a partial involvement of temporal (T5 electrode) and parietal lobes (P3 and P4). Differences between MCI and SCI were revealed during resting state mainly by the spectral parameters α2 and IAF peak, both related to the activity in alpha band. Group-related variations in these measures revealed that the MCI group had significantly lower median values for the IAF peak in the F4, Fz, C4 and Cz channels as well as it had lower α2 values in T5, C3, C4, P3 and P4. The β exponent too revealed changes associated with EEG waves alterations in SCI compared to MCI: significantly lower β exponent values in F3 and F7 were found in the latest. These differences between MCI and SCI in the analysis of single channel measures were consistent with those revealed by the analysis of the measures averaged on different regions of the scalp. The α2 values were lower for MCI patients in the left temporal, central and parietal regions, and the β exponent decreased in the MCI group compared to the SCI one in the left frontal regions. Nevertheless, the IAF peak was significantly different between the two groups only in the central region and not in the frontal ones, as it may be expected from the analysis of single channel measures.

On the other hand, during the memorization task, differences concerned primarily CS and SCI and were limited to the parietal lobe. The only exception was the β1 parameter calculated in the T6 channel, which had significantly lower values for MCI with respect to CS. The parameters revealing significant differences between CS and SCI were β2 and γ for the spectral analysis, and FD from a nonlinear point of view. All the changes in these parameters detected higher values for CS subjects with respect to those for SCI ones and were also confirmed in the analysis of the measures averaged on brain regions.

Table 3. Median values (with 25th and 75th percentiles) of the linear and non linear parameters, calculated for each channels and grouped channel in scalp regions, that showed significant differences among the CS, MCI and SCI groups, evaluated in Resting (R) or Memorization (M) testing condition, and p-values concerning the comparison for each pair of groups.

	Parameter	Test	CS	SCI	MCI	CS vs SCI	CS vs MCI	MCI vs SCI
Chan	***Linear***							
F4	*IAF peak*	R	9.02 (8.55-10.90)	8.27 (7.43-9.36)	9.52 (9.09-10.14)	n.s.	n.s.	0.04
Fz	*IAF peak*	R	9.03 (8.55-10.38)	7.88 (7.50-8.73)	9.48 (9.08-11.19)	n.s.	n.s.	0.04
Cz	*IAF peak*	R	8.67 (8.31-10.08)	8.25 (7.84-9.61)	10.16 (9.12-10.99)	n.s.	n.s.	0.003
C4	*IAF peak*	R	8.70 (9.31-10.08)	8.27 (7.73-9.84)	9.91 (9.22-12.06)	n.s.	n.s.	0.003
T5	*α2*	R	0.080 (0.067-0.143)	0.178 (0.100-0.231)	0.075 (0.053-0.099)	n.s.	n.s.	0.009
C3	*α2*	R	0.077 (0.070-0.106)	0.107 (0.085-0.154)	0.063 (0.044-0.104)	n.s.	n.s.	0.04
C4	*α2*	R	0.063 (0.055-0.095)	0.116 (0.086-0.160)	0.050 (0.040-0.086)	n.s.	n.s.	0.05
P3	*α2*	R	0.081 (0.072-0.145)	0.182 (0.107-0.228)	0.066 (0.061-0.088)	n.s.	n.s.	0.004
P4	*α2*	R	0.083 (0.063-0.135)	0.188 (0.115-0.263)	0.080 (0.069-0.094)	n.s.	n.s.	0.04
T6	*β1*	M	0.174 (0.128-0.197)	0.138 (0.078-0.213)	0.106 (0.080-0.125)	n.s.	0.020	n.s.
P4	*β2*	M	0.120 (0.094-0.164)	0.077 (0.038-0.084)	0.075 (0.064-0.106)	0.014	n.s.	n.s.
Pz	*β2*	M	0.111 (0.087-0.144)	0.072 (0.033-0.085)	0.061 (0.052-0.098)	0.014	n.s.	n.s.
P3	*γ*	M	0.154 (0.090-0.208)	0.059 (0.024-0.092)	0.110 (0.056-0.187)	0.009	n.s.	n.s.
Pz	*γ*	M	0.148 (0.077-0.172)	0.066 (0.020-0.076)	0.085 (0.048-0.153)	0.014	n.s.	n.s.
Chan	***Non Linear***							
F3	*β exponent*	R	1.20 (0.92-1.67)	1.61(1.21-1.86)	0.87 (0.74-1.38)	n.s.	n.s.	0.04
F7	*β exponent*	R	1.33 (0.98-1.53)	1.67 (1.38-1.91)	0.97 (0.75-1.20)	n.s.	n.s.	0.004
P3	*FD*	M	1.70 (1.65-1.78)	1.58 (1.47-1.61)	1.67 (1.52-1.81)	0.004	n.s.	n.s.
P4	*FD*	M	1.71 (1.64-1.81)	1.53 (1.45-1.60)	1.62 (1.51-1.74)	0.009	n.s.	n.s.
Pz	*FD*	M	1.66 (1.63-1.75)	1.53 (1.44-1.59)	1.62 (1.49-1.68)	0.002	n.s.	n.s.
Reg.	***Linear***							
C	*IAF peak*	R	8.92 (8.56-10.03)	8.80 (7.61-9.76)	10.15 (9.40-11.02)	n.s.	n.s.	0.04
LT	*α2*	R	0.074 (0.071-0.128)	0.140 (0.103-0.188)	0.068 (0.045-0.098)	n.s.	n.s.	0.009
C	*α2*	R	0.068 (0.064-0.102)	0.107 (0.087-0.157)	0.063 (0.042-0.087)	n.s.	n.s.	0.05
P	*α2*	R	0.074 (0.069-0.142)	0.184 (0.109-0.242)	0.079 (0.064-0.113)	n.s.	n.s.	0.04
P	*β2*	M	0.114 (0.097-0.155)	0.080 (0.037-0.085)	0.077 (0.062-0.100)	0.013	0.02	n.s.
P	*γ*	M	0.166 (0.082-0.215)	0.072 (0.023-0.079)	0.096 (0.053-0.177)	0.021	n.s.	0.012
Reg.	***Non Linear***							
LF	*β exponent*	R	1.44 (0.88-1.59)	1.57 (1.27-1.78)	1.02 (0.80-1.25)	n.s.	n.s.	0.012
P	*FD*	M	1.67 (1.65-1.78)	1.56 (1.45-1.59)	1.62 (1.51-1.73)	0.006	n.s.	n.s.

3.3. Comparison of EEG features between resting state and cognitive task

Differences between resting state and memorization task were analysed, within every single group, considering only regionally averaged parameters. Regional differences were considered more reliable than those possibly present in single channel measures because they reasonably reflected a local trend and they were more unlikely to be due to chance. Table 5 shows, for each parameter and each scalp region, the p-values of the Wilcoxon signed rank test performed to compare data during cognitive stimulation and resting state within each group of subjects. The parameters θ and IAF peak are not reported because they do not show significant variations in any of the three groups of subjects between resting state and cognitive task.

Table 4. P-values of the Wilcoxon signed rank test performed to compare, for each group of subjects (CS, MCI and SCI), parameters calculated during the memorization task and resting state, according to the scalp region.

Parameter	Group	Scalp region						
		RF	LF	RT	LT	C	P	O
	CS	n.s.	0.047	n.s.	n.s.	n.s.	0.016	0.047
Δ	SCI	0.047	0.047	n.s.	n.s.	n.s.	n.s.	n.s.
	MCI	0.014	n.s	n.s.	0.049	0.027	n.s.	n.s.
	CS	0.031*	0.047*	0.047*	0.016*	0.031*	0.031*	0.031*
α1	SCI	0.031*	0.016*	n.s.	n.s.	n.s.	n.s.	n.s.
	MCI	0.002*	0.002*	0.006*	0.010*	0.002*	0.002*	0.002*
	CS	0.047*	0.016*	n.s.	n.s.	n.s.	n.s.	n.s.
α2	SCI	0.016*	0.031*	0.016*	n.s.	0.016*	n.s.	0.016
	MCI	0.006*	0.004*	n.s.	n.s.	n.s.	n.s.	n.s.
	CS	0.031*	0.031*	n.s.	n.s.	n.s.	n.s.	n.s.
β1	SCI	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MCI	0.027*	0.004*	n.s.	n.s.	n.s.	n.s.	n.s.
	CS	n.s.	n.s.	n.s.	0.016	n.s.	n.s.	0.047
β2	SCI	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MCI	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	CS	n.s.	n.s.	n.s.	n.s.	0.047	0.031	n.s.
γ	SCI	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MCI	n.s.	n.s.	n.s.	n.s.	n.s.	0.049	n.s.
	CS	n.s.	0.031	n.s.	n.s.	n.s.	n.s.	n.s.
Total Power	SCI	0.016	0.047	n.s.	n.s.	n.s.	n.s.	n.s.
	MCI	0.002	0.006	n.s.	n.s.	0.014	n.s.	0.020
Non linear								
	CS	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
β exponent	SCI	n.s.	0.016	n.s.	n.s.	n.s.	n.s.	n.s.
	MCI	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	CS	n.s.	n.s.	n.s.	n.s.	n.s.	0.031	0.031
FD	SCI	n.s.	0.047	n.s.	n.s.	n.s.	n.s.	n.s.
	MCI	n.s.	n.s.	n.s.	n.s.	n.s.	0.049	0.037

Note: * = parameter value of resting state higher than of memorization task

In the CS group, changes between the baseline and the cognitive task mainly pertained to frontal regions, with significant variations for the α1, α2, β1 parameters and, limited to the left hemisphere, for the total power and the δ parameters. During the cognitive task α1, α2 and β1 waves were attenuated with respect to resting state, while total power increased. Significant changes of the α1 involved all the considered areas and not only the frontal one, while EEG alterations in the parietal and occipital areas were detected also by the δ, FD, and, partially, by the β2 and γ, which all increased during the mental task. β2 and γ, in addition to α1, were the only parameters that had significantly different values in the central and temporal regions.

In contrast to the CS group, in MCI group significant differences between the two experimental conditions were not localized in the frontal area, but rather spread to all the scalp regions. Like the CS group, during the cognitive task α1, α2 and β1 values were significantly lower in the frontal areas, as well as FD and γ increased compared to baseline in the parietal and occipital lobes. In the frontal, central and occipital regions memorization was associated with a significant enhancement of the total power value. Moreover, δ

significantly increased not only in the frontal lobe, but also in the left temporal and central regions.
SCI group presented EEG changes between the two tasks mainly limited to the frontal area, but they were revealed by partially different parameters compared to CS group. Changes determined by the memorization task in both right and left frontal regions were stated by an increase in the δ and in the total power values and, as it happened also for the other two groups, by an attenuation of α1 and α2 waves. Additional task-related alterations in the frontal region concerned, in the left hemisphere, the β exponent and the FD, with a decrease and an increase, respectively, compared to resting state. Unlike the other two groups, the α1 attenuations were limited to the frontal region, while α2 lowered in all areas, except for the left temporal and the parietal ones.
The parameters θ and IAF peak were not affected by variations between resting state and cognitive task in any of the three groups of subjects.

4. DISCUSSION

In the present study, we analyzed EEGs recorded during resting state and cognitive stimulation in SCI, MCI and normal controls, using a combination of parameters extracted from single channels and from brain regions, with both linear and non-linear approach.
Resting state was found to be a good condition to differentiate between MCI and SCI (mainly by using the spectral parameters α2 and IAF peak, both related to the activity in alpha band), although broad overlap between the values in CS and both in SCI and in MCI was present. On the other hand the cognitive task allowed assessing differences between CS and SCI (β2 and γ from spectral analysis, FD from nonlinear approach) while numerous overlaps between MCI and both SCI and CS were displayed. The overlapping produced P/values close to the limit of significance.
A decrease in α1 power for both MCI and SCI compared to CS was also found even if not statistically relevant in our population; this result is consistent with literature and can be considered a key feature of cognitive impairment [53].
The use of cognitive stimulation allowed to detect the main findings of this study.
Delayed recall performance, which can be considered as the oral version of the mental recall task during the second EEG recording, appeared to be below normal values (ES=0) for 45% (5/11) MCI subjects and for no one of SCI and CS subjects; at lower limit of normal values (ES=1) for 18% (2/11) MCI subjects, for 12.5% (1/8) SCI subjects and for no one of control group (Table 2). These results are in line with the group classification in the recruitment phase, as we would have expected from neuropsychological testing.
Comparing EEG features between resting state and cognitive task, α1 and α2 were found attenuated during the cognitive task compared to resting state in all three groups. Previous studies have demonstrated that alpha rhythm desynchronization (or power decrease), particularly upper alpha band, is required for good memory functioning, both in encoding and in retrieval [54], [55], while an increase in α2 power reflects a stop of information processing [56]. We can therefore assume that all subjects were really performing the task. As during the second EEG recording a mental test was performed, in order to minimize the artifacts, we consider the oral recall after EEG registration a proof of the subjects participation to the mental task during EEG2. A decrease of α1 and α2 power was found predominantly in the frontal regions in SCI and control group, while it spreads in all the scalp regions in MCI group. Previous functional MRI studies have demonstrated that the desynchronization related to a successful encoding of new items typically involves temporo-parietal memory-related networks, the same areas which are primarily damaged in AD [57], [58]. However, all these studies were based on semantic encoding task, while our EEG were acquired during free recall task that requires the involvement of the frontal area [59] ; this could explain our findings for SCI and CS. In MCI patients we have hypothesized that different brain networks need to be activated to perform the same recall task, in order to compensate for the difficulty of encoding [60]. Conversely, since SCI behave as normal control on testing by definition, we supposed they do not need to compensate during the task.
The use of nonlinear measures to study EEG alterations determined by cognitive impairment revealed interesting results. This approach, based on the principles of nonlinear dynamics and deterministic chaos, has been effectively applied to EEG in subjects with cognitive decline [61] and the addition of nonlinear EEG measures to the classical ones has also shown to add valuable complementary information in EEGs characterization [29], [31], [62], [63]. Nevertheless, with respect to standard spectral measures, relationships between different nonlinear EEG parameters and cognitive decline are less well-established, also in relation to their physiological meaning. A decreased complexity of EEG patterns in entire brain regions in AD patients is generally considered one of the major effects of AD on EEG [17], but there is still lack of detailed information concerning the impact of early stages of cognitive decline on different nonlinear parameters. In our work we found that, compared to normal elderly, EEG of SCI subjects during the cognitive task showed a decreased complexity, revealed by lower values of FD, mainly located in the parietal region. This finding is in line with the reduced complexity showed in EEG patterns of AD patients in previous studies [17]; in our study it appears that FD could be a good parameter to differentiate between SCI and CS during cognitive stimulation, with main differences located in the parietal regions (primarily involved in the degenerative process of this disease). We could therefore hypothesize that this finding is a possible early hallmark of the disease.
Furthermore, a reduced complexity was found also in MCI group, even if it was not significantly different from FD values in CS and SCI. We would underline

that the small sample size of the study population could have influenced the results producing large confidence intervals as well as many p-values close to the limit of significance for some parameters (as shown in Tables 3 and 4).
The hypothesis that EEG features can be used in the discrimination of normal elderly, MCI and AD subjects during resting state has already been tested [11], but the characterization of SCI subjects and the use of EEG measures during a cognitive task may be considered as novel aspects of this work. The discrimination of SCI subjects from CS during a memory task is encouraging and unveils the potential of EEG as an useful, cheap and non-invasive instrument for early-stage detection of cognitive impairment.

5. CONCLUSIONS

This study concerning MCI and SCI subjects drew attention on the importance of the non-linear approach in EEG analysis and on the potential role of cognitive task conditions in determining EEG alterations at the very early stages of AD, even in patients with subjective memory complaints.
The main finding of this study is that the use of a free recall cognitive task, requiring the involvement of frontal areas, determined an attenuation of α power restricted to frontal regions in SCI patients, compared to a more diffused decrease in anterior and posterior regions in MCI subjects. We could therefore assume that MCI patients need to compensate for the difficulty of encoding by activating different brain networks to perform the same recall task.
Moreover, EEG of SCI subjects during the cognitive task compared to normal elderly showed a decreased complexity, which could be a possible early hallmark of the disease, worthy of further investigation.
We suggest that this analysis may be applied in a clinical context as a diagnostic and prognostic tool in subjects with complaining of initial cognitive impairment, considering that EEG characterization was carried out with simple protocol on short EEG epochs. Further studies are needed to confirm the statistical significance of these results in an enlarged study population.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the local ethics committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants released their informed consent

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are basis of this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We are grateful to the technicians Luca Mantoani, Antonio Draisci, Giuseppe Romano, Mauro Semenic, Elizabeth Boarini, Walter Calligaris.

REFERENCES

[1] Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, *et al.* Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement.;7(3):280–92 (2011).
[2] Reisberg B, Gauthier S. Current evidence for subjective cognitive impairment (SCI) as the pre-mild cognitive impairment (MCI) stage of subsequently manifest Alzheimer's disease. Int Psychogeriatr. 20(1):1–16 (2008).
[3] Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, *et al.* Mild cognitive impairment. Lancet Lond Engl. 367:1262–70 (2006).
[4] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, *et al.* The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement J Alzheimers Assoc. 7(3):270–9 (2011).
[5] Hampel H, Bürger K, Teipel SJ, Bokde ALW, Zetterberg H, Blennow K. Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. Alzheimers Dement J Alzheimers Assoc. 4(1):38–48 (2008).
[6] Galluzzi S, Geroldi C, Amicucci G, Bocchio-Chiavetto L, Bonetti M, Bonvicini C, *et al.* Supporting evidence for using biomarkers in the diagnosis of MCI due to AD. J Neurol. 260(2):640–50 (2013).
[7] Arendt T. Synaptic degeneration in Alzheimer's disease. Acta Neuropathol (Berl). 118(1):167–79 (2009).
[8] Takahashi RH, Capetillo-Zarate E, Lin MT, Milner TA, Gouras GK. Co-occurrence of Alzheimer's disease ß-amyloid and τ pathologies at synapses. Neurobiol Aging. 31(7):1145–52 (2010).
[9] Cook IA, Leuchter AF. Synaptic dysfunction in Alzheimer's disease: clinical assessment using quantitative EEG. Behav Brain Res. 78(1):15–23 (1996).
[10] Sorg C, Riedl V, Perneczky R, Kurz A, Wohlschlager AM. Impact of Alzheimer's Disease on the Functional Connectivity of Spontaneous Brain Activity. Curr Alzheimer Res. 6(6):541–53 (2009).

[11] Vecchio F, Babiloni C, Lizio R, Fallani FDV, Blinowska K, Verrienti G, *et al*. Resting state cortical EEG rhythms in Alzheimer's disease: toward EEG markers for clinical applications: a review. Suppl Clin Neurophysiol. 62:223–36 (2013).

[12] Babiloni C, Binetti G, Cassetta E, Dal Forno G, Del Percio C, Ferreri F, *et al*. Sources of cortical rhythms change as a function of cognitive impairment in pathological aging: a multicenter study. Clin Neurophysiol Off J Int Fed Clin Neurophysiol. 117(2):252–68 (2006).

[13] Kwak YT. Quantitative EEG findings in different stages of Alzheimer's disease. J Clin Neurophysiol Off Publ Am Electroencephalogr Soc. 23(5):456–61(2006).

[14] Lehmann C, Koenig T, Jelic V, Prichep L, John RE, Wahlund L-O, *et al.* Application and comparison of classification algorithms for recognition of Alzheimer's disease in electrical brain activity (EEG). J Neurosci Methods. 161(2):342–50 (2007).

[15] Luckhaus C, Grass-Kapanke B, Blaeser I, Ihl R, Supprian T, Winterer G, *et al*. Quantitative EEG in progressing vs stable mild cognitive impairment (MCI): results of a 1-year follow-up study. Int J Geriatr Psychiatry. 23(11):1148–55 (2008).

[16] Rossini PM, Buscema M, Capriotti M, Grossi E, Rodriguez G, Del Percio C, *et al*. Is it possible to automatically distinguish resting EEG data of normal elderly vs. mild cognitive impairment subjects with high degree of accuracy? Clin Neurophysiol. 119(7):1534–45 (2008).

[17] Dauwels J, Vialatte F, Cichocki A. Diagnosis of Alzheimer's disease from EEG signals: where are we standing? Curr Alzheimer Res. 7(6):487–505 (2010).

[18] Prichep LS, John ER, Ferris SH, Rausch L, Fang Z, Cancro R, *et al*. Prediction of longitudinal cognitive decline in normal elderly with subjective complaints using electrophysiological imaging. Neurobiol Aging. 27(3):471–81(2006).

[19] Prichep LS. Quantitative EEG and electromagnetic brain imaging in aging and in the evolution of dementia. Ann N Y Acad Sci. 1097:156–67 (2007).

[20] Antila K, Lötjönen J, Thurfjell L, Laine J, Massimini M, Rueckert D, *et al.* The PredictAD project: development of novel biomarkers and analysis software for early diagnosis of the Alzheimer's disease. Interface Focus. 3(2):20120072 (2013).

[21] Poil SS, de Haan W, van der Flier WM, Mansvelder HD, Scheltens P, Linkenkaer-HansenK. Integrative EEG biomarkers predict progression to Alzheimer's disease at the MCI stage. Front. Aging Neurosci. 5: 58 (2013).

[22] Moretti DV, Frisoni GB, Fracassi C, Pievani M, Geroldi C, Binetti G, *et al.* MCI patients' EEGs show group differences between those who progress and those who do not progress to AD, Neurobiol. Aging. 32 (4): 563–571(2011).

[23] Missonnier P, Deiber MP, Gold G, Herrmann FR, Millet P, Michon A, *et al.* Working memory load-related electroencephalographic parameters can differentiate progressive from stable mild cognitive impairment. Neuroscience.150 (2): 346–356 (2007).

[24] Rossini PM, Del Percio C, Pasqualetti P, Cassetta E, Binetti G, Dal Forno G *et al.* Conversion from mild cognitive impairment to Alzheimer's disease is predicted by sources and coherence of brain electroencephalography rhythms. Neuroscience. 143(3): 793–803 (2006).

[25] Babiloni C, Visser PJ, Frisoni G, De Deyn PP, Bresciani L, Jelic V, *et al*. Cortical sources of resting EEG rhythms in mild cognitive impairment and subjective memory complaint. Neurobiol Aging. 31(10):1787–98 (2010).

[26] Alexander DM, Arns MW, Paul RH, Rowe DL, Cooper N, Esser AH *et al.* EEG markers for cognitive decline in elderly subjects with subjective memory complaints. J. Integr. Neurosci. 5(1): 49–74 (2006).

[27] Schmidt MT, Anghinah R, Basile LF, Forlenza O, Gattaz WF. EEG alpha peak frequency analysis during memorizing of figures in patients with mild cognitive impairment. Arq Neuropsiquiatr. 67(2B):432–8 (2009).

[28] Accardo A, Affinito M, Carrozzi M, Bouquet F. Use of the fractal dimension for the analysis of electroencephalographic time series. Biol Cybern. 77(5):339–50 (1997).

[29] Carrozzi M, Accardo A, Bouquet F. Analysis of sleep-stage characteristics in full-term newborns by means of spectral and fractal parameters. Sleep. 27(7):1384–93 (2004).

[30] Stam CJ. Nonlinear dynamical analysis of EEG and MEG: review of an emerging field. Clin Neurophysiol Off J Int Fed Clin Neurophysiol. 116(10):2266–301(2005).

[31] Accardo A, Cusenza M, Monti F. Linear and non-linear parameterization of EEG during monitoring of carotid endarterectomy. Comput Biol Med. 39(6):512–8 (2009).

[32] Folstein MF, Folstein SE, McHugh PR. «Mini-mental state». A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 12(3):189–98 (1975).

[33] Measso G, Cavarzeran F, Zappalà G, Lebowitz BD, Crook TH, Pirozzolo FJ, *et al*. The mini□mental state examination: Normative study of an Italian random sample. Dev Neuropsychol. 9(2):77–85 (1993).

[34] Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, *et al*. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 53(4):695–9 (2005).

[35] Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry. 23:56–62 (1960).

[36] Novelli G, Papagno C, Capitani E, Laiacona M, Al E. Tre test clinici di memoria verbale a lungo termine: Taratura su soggetti normali. Arch Psicol Neurol Psichiatr. 47: 278-96 (1986).

[37] Spinnler, Tognoni. Standardizzazione e taratura italiana di test neuropsicologici. Ital J Neurol Sci. 8(supplement 8):8–120 (1987).
[38] Reitan RM. The relation of the Trail Making Test to organic brain damage. J Consult Psychol. 19(5):393–4 (1955).
[39] Giovagnoli AR, Del Pesce M, Mascheroni S, Simoncelli M, Laiacona M, Capitani E. Trail making test: normative values from 287 normal adult controls. Ital J Neurol Sci. 17(4):305–9 (1996).
[40] Rey A. L'examen psychologique dans les cas d'encéphalopathie traumatique. (Les problems.). Arch Psychol. 28: 286–340 (1941).
[41] Osterrieth PA. Le test de copie d'une figure complexe; contribution à l'étude de la perception et de la mémoire. Arch Psychol. 30: 286–356 (1944).
[42] Caffarra P, Vezzadini G, Dieci F, Zonato F, Venneri A. Rey-Osterrieth complex figure: normative values in an Italian population sample. Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol. 22(6):443–7 (2002).
[43] Rey A. L'examen clinique en psychologie. Presses Universitaires de France, Paris (1958).
[44] Carlesimo GA, Caltagirone C, Gainotti G. The Mental Deterioration Battery: normative data, diagnostic reliability and qualitative analyses of cognitive impairment. The Group for the Standardization of the Mental Deterioration Battery. Eur Neurol. 36(6):378–84 (1996).
[45] Knopman DS, Ryberg S. A verbal memory test with high predictive accuracy for dementia of the Alzheimer type. Arch Neurol. 46(2):141–5 (1989).
[46] Gainotti G, Marra C, Villa G, Parlato V, Chiarotti F. Sensitivity and specificity of some neuropsychological markers of Alzheimer dementia. Alzheimer Dis Assoc Disord. 12(3):152–62 (1998).
[47] Ricci M, Graef S, Blundo C, Miller LA. Using the Rey Auditory Verbal Learning Test (RAVLT) to Differentiate Alzheimer's Dementia and Behavioural Variant Fronto-Temporal Dementia. Clin Neuropsychol. 26(6):926–41(2012).
[48] Nunez PL, Srinivasan R. Electric Fields of the Brain: The Neurophysics of EEG. Oxford University Press. pp.629 (2006).
[49] Klimesch W. EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. Brain Res Brain Res Rev. 29(2–3):169–95 (1999).
[50] Pritchard WS. The brain in fractal time: 1/f-like power spectrum scaling of the human electroencephalogram. Int J Neurosci. 66(1–2):119–29 (1992).
[51] Higuchi T. Approach to an irregular time series on the basis of the fractal theory. Phys Nonlinear Phenom. 31(2):277–83(1988).
[52] Fornasa E, Accardo A, Mazzon G, Monti F. EEG Analysis in Resting State and during a Memorization Task in Mild Cognitive Impairment and Subjective Cognitive Impairment. In: Braidot A., Hadad A. (eds) VI Latin American Congress on Biomedical Engineering CLAIB 2014, Paraná, Argentina 29, 30 & 31 October 2014. IFMBE Proceedings,49: 635-638. Springer, Cham (2015)
[53] Başar E, Güntekin B. Review of delta, theta, alpha, beta, and gamma response oscillations in neuropsychiatric disorders. Suppl Clin Neurophysiol. 62:303–41(2013).
[54] Hanslmayr S, Staudigl T, Aslan A, Bäuml K-H. Theta oscillations predict the detrimental effects of memory retrieval. Cogn Affect Behav Neurosci. 10(3):329–38 (2010).
[55] Staudigl T, Hanslmayr S, Bäuml K-HT. Theta oscillations reflect the dynamics of interference in episodic memory retrieval. J Neurosci Off J Soc Neurosci. 30(34):11356–62 (2010).
[56] Moretti DV, paternicò donata, Binetti G, Zanetti O, Frisoni giovanni b. EEG upper/low alpha frequency power ratio relates to temporo-parietal brain atrophy and memory performances in mild cognitive impairment. Front Aging Neurosci. 5:63 (2013).
[57] Sperling RA, Dickerson BC, Pihlajamaki M, Vannini P, LaViolette PS, Vitolo OV, et al. Functional Alterations in Memory Networks in Early Alzheimer's Disease. NeuroMolecular Med. 12(1):27–43 (2010).
[58] Chhatwal JP, Sperling RA. Functional MRI of mnemonic networks across the spectrum of normal aging, mild cognitive impairment, and Alzheimer's disease. J Alzheimers Dis JAD. 31 Suppl 3:S155-167 (2012).
[59] Dhanjal NS, Wise RJS. Frontoparietal cognitive control of verbal memory recall in Alzheimer's disease. Ann Neurol. 76(2):241–51 (2014).
[60] Jiang Z, Zheng L. Inter- and intra-hemispheric EEG coherence in patients with mild cognitive impairment at rest and during working memory task. J. Zhejiang Univ. Sci. B. 7(5):357–364 (2006).
[61] Jeong J, Chae JH, Kim SY, Han SH. Nonlinear dynamic analysis of the EEG in patients with Alzheimer's disease and vascular dementia. J Clin Neurophysiol Off Publ Am Electroencephalogr Soc. 18(1):58–67 (2001).
[62] Mayer-Kress G, Layne SP. Dimensionality of the human electroencephalogram. Ann N Y Acad Sci. 504:62–87 (1987).
[63] Pritchard WS, Duke DW, Coburn KL, Moore NC, Tucker KA, Jann MW, *et al*. EEG-based, neural-net predictive classification of Alzheimer's disease versus control subjects is augmented by non-linear EEG measures. Electroencephalogr Clin Neurophysiol. 91(2):118–30 (1994).