



## Subclinical epileptiform activity accelerates the progression of Alzheimer's disease: A long-term EEG study



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See Editorial, pages 1961–1963

### ARTICLE INFO

#### Article history:

Accepted 29 March 2021

Available online 8 May 2021

#### Keywords:

Alzheimer's disease

Epilepsy

Epileptiform discharge

Electroencephalography

Neuropsychology

### HIGHLIGHTS

- Epileptiform discharges are presented in half of Alzheimer patients without epileptic seizures.
- Alzheimer patients with epileptiform discharges have significantly lower memory performance.
- Epileptiform discharges associate with 1.5-times faster cognitive decline in a prospective follow-up.

### ABSTRACT

**Objective:** While many studies suggest that patients with Alzheimer's disease have a higher chance for developing epileptic seizures, only a few studies are available examining independent epileptic discharges. The major aims of our study was to determine the prevalence of subclinical epileptiform activity (SEA) in AD compared to healthy elderly controls with the hypothesis that SEA is more frequent in AD than in cognitively normal individuals. Another aim was to analyze the effect of baseline SEA captured with electroencephalography on the progression of the disease with longitudinal cognitive testing.

**Methods:** We investigated 52 Alzheimer patients with no history of epileptic seizures and 20 healthy individuals. All participants underwent a 24-hour electroencephalography, neurology, neuroimaging and neuropsychology examination. Two independent raters analyzed visually the electroencephalograms and both raters were blind to the diagnoses. Thirty-eight Alzheimer patients were enrolled in a 3-year long prospective follow-up study with yearly repeated cognitive evaluation.

**Results:** Subclinical epileptiform discharges were recorded significantly ( $p:0.018$ ) more frequently in Alzheimer patients (54%) than in healthy elderly (25%). Epileptiform discharges were associated with lower performance scores in memory. Alzheimer patients with spikes showed 1.5-times faster decline in global cognitive scores than patients without ( $p < 0.001$ ). The decline in cognitive performance scores showed a significant positive correlation with spike frequency ( $r:+0.664$ ;  $p < 0.001$ ).

**Conclusions:** Subclinical epileptiform activity occurs in half of Alzheimer patients who have never suffered epileptic seizures. Alzheimer patients with subclinical epileptiform activity showed accelerated cognitive decline with a strong relation to the frequency and spatial distribution (left temporal) of spikes. **Significance:** Our findings suggest the prominent role of epileptiform discharges in the pathomechanism of Alzheimer's disease which might serve as potential therapeutic target.

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## 1. Introduction

Alzheimer’s disease (AD) is the major cause of cognitive decline creating ponderous economic burden on the aging societies. It is well known that some factors modify the progression, e.g. the onset of cognitive decline, an advanced disease stage at the time of diagnosis, educational background, female gender, and comorbid cardiovascular disease (Ito et al., 2011; Devanand et al., 2013; Doody et al., 2010).

A recently recognized concomitant condition is epilepsy. In animal models of AD, epileptic seizures are common (Palop et al., 2008; Palop and Mucke, 2010). Also, neuropathology studies have identified many similarities between AD and temporal lobe epilepsy such as the presence of amyloid plaques, tau neurofibrils, hippocampal sclerosis and hippocampal hyperexcitability (Puvanna et al., 2006; Riascos et al., 2014; Tai et al., 2016). Growing body of evidence suggests that AD patients have an increased risk to develop epileptic seizures and seizures associate with worse cognitive functioning (Horváth et al., 2016; Palop and Mucke, 2009). In addition to taking into account the cognitive harm of clinical seizures, modern epileptology has recognized the negative impact of epileptiform discharges on the cognitive performance of epilepsy patients Ung et al., 2017).

Considering the harm of epileptic discharges on cognitive functions in epilepsy, it is intriguing to investigate the effect of epileptiform activity in AD patients without clinical seizures (subclinical epileptiform activity- SEA) (Fisher et al., 2014). SEA could be observed among healthy individuals (Santoshkumar et al., 2009); however, the physiological background of the phenomenon (as a potential marker of cortical hyperexcitability or an indicator of an undiagnosed neurological disorder) is unclarified (McLachlan and Luba, 2002). Many early studies demonstrated that SEA might serve as a predictive marker for the further development of epileptic seizures (Saito et al., 1987), however, it was not clarified with sensitive neurophysiological techniques or meta-analysis (So 2010). Recent studies demonstrated that SEA might predict poor outcome in acute vascular events (Tabaeizadeh et al., 2020), associate with more severe symptoms in autism spectrum disorders (Mulligan and Trauner, 2014) and predict epileptic seizures in children with attention-deficit hyperactivity disorders (Richer et al., 2002). There is clearly a scientific trend suggesting that SEA might have a role in the development of many disorders affecting cognitive functions (Horvath et al., 2020). It is confirmed by a recent human study that SEA might impair the cognitive functions of AD patients (Vossel et al., 2016); however, these results have not been replicated yet.

The aim of our prospective study was to determine the prevalence of SEA in AD compared to healthy elderly controls with the hypotheses that 1, SEA is more frequent in AD than in cognitively normal individuals, and 2, baseline SEA captured with electroencephalography (EEG) associate with faster progression of the disease. We also aimed to establish the link between the temporal and spatial distribution of epileptiform spiking and cognitive decline.

## 2. Materials and methods

### 2.1. Participants

We studied 80 AD patients with clinically typical, predominantly memory-associated symptoms who met the diagnostic criteria of the National Institute of Aging- Alzheimer’s Association (NIA-AA) for probable AD (McKhann et al., 2011) and 20 cognitively healthy controls (HC) in the Department of Neurology of National Institute of Clinical Neurosciences in Budapest and in

the Department of Neurology of Kaposi Mór County Hospital in Kaposvár, Hungary between 2015 and 2019. All subjects were native Hungarians.

We collected medical history including collateral data and medical records for excluding those patients with epileptic seizures or seizure like events. Participants who had epileptic seizures or elevated risk for epileptic seizures including prior infection of central nervous system, clinically significant brain lesions (stroke, severe periventricular white matter disease, white matter infarcts), head trauma with loss of consciousness, demyelinating conditions, hydrocephalus, untreated vitamin B12 deficiency or hypothyroidism, syphilis or HIV infection, major depression, schizophrenia, electroconvulsive therapy, renal insufficiency, liver disease, significant systemic medical illness, alcohol or substance dependency, psychoactive drugs affecting cognitive functions except anti-dementia medication were excluded from the current analysis (n:21). Furthermore, 7 patients with major depression measured with neuropsychological tests were also excluded (Fig. 1).

Data of 52 AD patients were analyzed in the prevalence analysis (studies at year 0). Data from 32 patients were used in a previous publication of our group (Horvath et al., 2018). In that study, only the demographic characteristics were analyzed to compare AD patients with SEA to AD patients with epileptic seizures.

We followed our patients for 3 years and repeated the same neuropsychology test battery each year. During the 3-year follow-up, we excluded 5 AD patients from the prospective analysis who in addition to AD suffered significant physical or mental illness potentially affecting cognitive functioning during the follow-up period. Four AD patients developed seizures during the follow-up period, therefore their data were excluded from the statistical analysis. Five more patients were lost to follow-up because of being unreachable. Finally, we analyzed the prospective data of the remaining 38 AD patients at the end of the 3rd year in the longitudinal analysis (Fig. 1).

### 2.2. Standard protocol approvals, registration, and phenotyping

The Hungarian Medical Research Council authorized our research (reference number: 024505/2015). We obtained informed

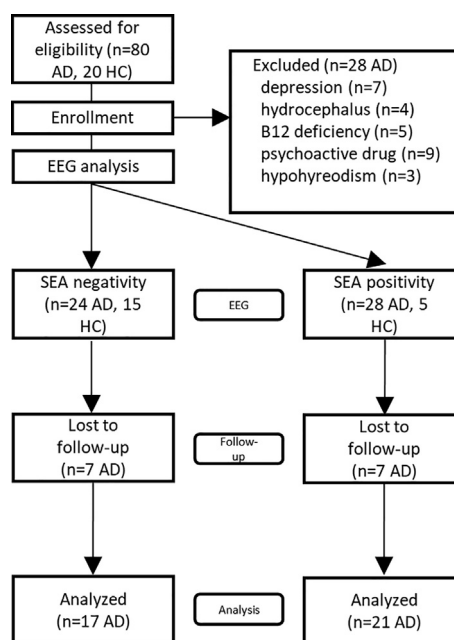


Fig. 1. FLOW-CHART OF PARTICIPANT RECRUITMENT AND ENROLLMENT. AD: Alzheimer’s disease; HC: healthy control; EEG: electroencephalography; SEA: subclinical epileptiform activity.

written consent from each participant. Surrogate consents were not required since all cases were mild so that all patients consented for themselves.

### 2.3. Clinical testing

The participants underwent detailed physical, neurological, and epileptological examination, as well as routine blood checks including thyroid functions and vitamin B12 level. All subjects had structural brain magnetic resonance imaging (MRI).

The controls had normal neurology status and neuropsychology scores, normal brain MRI and blood results, and they had no cognitive complaints.

### 2.4. Neuropsychological examination

The neuropsychological tests were carried out by trained neurologists or neuropsychologists in Hungarian language at the beginning of the study (year 0) and at the beginning of each upcoming year (year 1, year 2, year 3) within 335–395 days following the previous examination. We used the Hungarian version of Addenbrooke Cognitive Examination (ACE) (Stacho et al., 2002) as primary test battery because of its high sensitivity and specificity in the diagnosis of major neurocognitive disorders. ACE scores range from 0 to 100, higher score represents better cognitive performance. ACE measures six cognitive domains including orientation (spatial and temporal- e.g. identification of home city of current year), attention (e.g. counting), memory (anterograde, retrograde and delayed recall- e.g. memorization of an address or words), verbal fluency (category and letter- e.g. listing of animals), language (e.g. execution of written or oral orders) and visuo-spatial skills (e.g. clock drawing and copying of a cube or intersecting pentagons). ACE is an accurate tool in differentiating frontotemporal dementia and AD too, and it serves properly in the assessment of dementia severity since Mini-Mental State Examination Score (MMSE) can be extracted from the test data (Dudas et al., 2005). Scores in the MMSE range from 0 to 30, higher scores denote better cognitive performance. ACE also includes the ratio of verbal fluency and language skills divided by the scores of delayed recall memory and orientation (VLOM ratio). The normal value for VLOM ratio is 2.2–3.2, scores < 2.2 indicate frontotemporal type deficit while scores > 3.2 denote Alzheimer-type impairment.

Since anxiety and depression might compromise cognitive functions, we also recorded the Hungarian version of Spielberger State and Trait Anxiety Inventory (STAI) (Sipos and Sipos, 1983) and Beck Depression Inventory II (BDI-II) (Miklosi et al., 2011). Based on large samples, <45 value on STAI represents low level of anxiety in both the trait and state category (Horvath et al., 2016). Scores < 13 indicate minimal depression, the 14–19 range represents mild, the range 20–28 moderate and a score higher than 29 signalizes severe depression. To increase our diagnostic accuracy, patients with a STAI > 45 and a BDI II > 13 were not included in our analysis (possible indicators of major depression).

Controls had MMSE > 26 (Janka et al., 1989) Addenbrooke Cognitive Examination (ACE) score > 84 (Stacho et al., 2002), STAI < 45 (Sipos and Sipos, 1983), BDI II < 13 (Miklosi et al., 2011).

### 2.5. EEG examination

We performed 34-channel 24-hour long inpatient EEG recording (Micromed Morpheus, 10–20 electrode placement system) in all participants within 5 days following the neuropsychological and clinical testing at the beginning of the study (year 0). EEGs during the follow-up period were not repeated. Peri-orbital leads were applied to aid sleep staging. We used the following EEG settings: bipolar longitudinal montage, 10 microvolts/mm sensitivity,

30 mm/sec speed, 70 Hz low pass and 0.5 Hz high pass filter with 50 Hz notch filter on.

Subclinical epileptiform discharges were defined as paroxysmal EEG graphoelements (spikes or sharp waves) with 20–200 ms duration, with the disruption of background EEG activity, followed by slow waves (Noachtar and Rémi, 2009). Two independent raters analyzed visually the EEGs; a graphoelement was identified as epileptiform if both raters marked it so. Both raters were blind to the diagnoses. For avoiding misinterpretation of epileptic transients, we identified and excluded from the calculation the following variants: wicket spikes, occipital sharp transients of sleep, benign epileptiform transients in sleep, and rhythmic temporal theta series in superficial sleep. Based on these evaluations, participants were categorized into EEG positive (AD + SEA) and negative (AD-SEA) subgroups.

The number of spikes was visually counted. The average number of spikes/hours was calculated as the total number of spikes divided by the hours of recordings. The scalp distribution of SEA was analyzed both visually and with the application of automatic EEG software (Micromed SystemPLUS 98, Compumedics NeuroScan Curry 7). Recognition of spatial distribution of SEA was based on the largest electronegativity corresponding to scalp electrodes in the 10–20 electrode placement system as follows: frontal (Fp1, Fp2, F3, F4), frontocentral (Fz), central (C3, Cz, C4), centroparietal (Pz), frontotemporal (F7, F8), temporal (T3, T4, T5, T6), parietal (P3, P4) and occipital (O1, O2) electrodes.

### 2.6. Statistical analysis

IBM SPSS 20 software (<https://www.ibm.com/support/pages/ibm-spss-statistics-20-documentation>) was applied for statistical analysis. A recent study of Vossel et al. (Vossel et al., 2016) reported difference in MMSE change /year in AD + SEA patients (mean = 3.9, SD = 1.6) compared to AD-SEA patients (mean = 1.6, SD = 2.4). Based on their results and our power calculations the probability was equal or greater than 80% to find a significant ( $\alpha = 0.05$ ) difference between study groups in MMSE deterioration (in delta MMSE / year) with a sample size of 50. Drop of rate (27%) was higher than calculated. In pairwise comparisons, t-tests were used for continuous data with parametric distribution, while Mann-Whitney U-test was applied for data with non-parametric distribution. Distribution was analyzed with Shapiro-Wilk test. For pairwise comparisons of categorical variables, chi-square test was applied. Holm-Bonferroni method was applied for correction of multiple comparisons. Logistic regression with the age as a covariant was applied for comparison of SEA prevalence across AD patients and controls, since AD patients represented an older cohort.

Longitudinal changes in the neuropsychological data represented by ACE score and MMSE score (at the beginning, after 1-year, after 2-year and after 3-year follow up) across AD + SEA and AD-SEA patients were compared using repeated measure general linear model (r-GLM). Linear model was selected because Shapiro-Wilk test indicated normal distribution of ACE and MMSE data ( $p > 0.05$ ). Between subject factor was the presence (AD + SEA) or absence of epileptiform activity (AD-SEA), while the measured ACE and MMSE scores at 0 time point, at 1-year, at 2-year and at 3-year represented the within subject variable (dependent factor). Greenhouse-Geisser correction was applied to report p- and F-values for pairwise comparisons since Mauchly's test reported that sphericity could not be assumed ( $p < 0.05$ ). Tukey-test was applied for post-hoc analysis with Bonferroni correction to analyze changes between different time points. We report adjusted p-values for the multiple comparisons of 4 time points (0-, 1-, 2-, 3-year) and indicate significance where  $p < 0.0125$ .

Known progression modifying factors such as age at onset of cognitive decline, gender, education level (represented in years of education) and disease severity (represented in 0 timepoint MMSE score) were added as covariates to the model. Effect of these factors derived from the linear model was reported.  $P < 0.05$  represented statistically significant effect. Spearman-correlation was applied to measure the association between progression of cognitive decline and spike frequency.

All statistical analyses concerning EEG data were performed using the data derived from the long-term EEG recording performed at year 0.

### 2.7. Data availability

The data that support the findings of this study and not presented in this article are available on request from the corresponding author.

## 3. Results

### 3.1. Demographics and clinical characteristics at year 0

The AD group (n: 52) was older than the control group (n: 20) ( $p < 0.001$ ). Patients were in the mild or moderate phase of the disease. Neuropsychology indicated typical AD representation of cognitive impairment (deficit dominantly in orientation and episodic memory) in all patients (Table 1). MRIs were analyzed with visual inspection and in all patients showed characteristic bifrontal-bitemporal atrophy as well as hippocampal atrophy typical of AD.

### 3.2. Prevalence, spatial distribution and spike frequency of SEA in AD at year 0

We detected SEA in 54% of AD patients (28/52) and in 25% of HCs (5/20), the difference is significant ( $p:0.018$ ). SEA was detected predominantly over the temporal electrodes (23/28 patients = 82%) (Fig. 2). Temporal SEA lateralized predominantly to the left side (12/23 = 52%), while bitemporal (6/23 = 26%) and right temporal occurrence (5/23 = 22%) were less common (Fig. 2). In patients with SEA, spike frequency was 0.29–6.68 spikes/hour (in average 2.02 spikes/hour). The vast majority of spikes (92%) occurred dur-

**Table 1**  
Demographic and clinical parameters of participants.

Characteristic	Controls	AD patients	p-value
Number	20	52	-
Female sex (n: %)	9 (45%)	31 (59,6%)	0.346
Age (years, mean ± SD)	67.8 ± 4.8	75.5 ± 8	*0.01
Right handedness (%)	18 (90%)	48 (92.3%)	0.772
Number of years of education in median score (interquartile range)	12 (12–17)	12 (12–17)	0.142
MMSE median score (interquartile range)	28.5 (27.3–29)	20 (16–23)	*<0.001
ACE median score (interquartile range)	92.5 (89.3–94.8)	66 (56–74)	*<0.001
VLOM median ratio (interquartile range)	2.7 (2.5–3)	3.4 (3.2–4.1)	*<0.001

Statistical tests were Chi-square for sex and handedness, t-test for age and Mann-Whitney U-test for MMSE score, ACE score and VLOM ratio.

AD: Alzheimer’s disease, MMSE: Mini-Mental Score Examination, ACE: Addenbrooke Cognitive Examination, VLOM ratio: sum of verbal fluency and language scores divided by sum of orientation and delayed memory recall scores, SD: standard deviation. \*indicates significant differences ( $p < 0.05$ ).

ing sleep. Spikes appeared most frequently during stage 2 (31%) and stage 3 (34%) sleep, while 23% of spikes were detected in stage 1 sleep. Only 4% of spikes occurred during rapid eye movement (REM) sleep (Fig. 2).

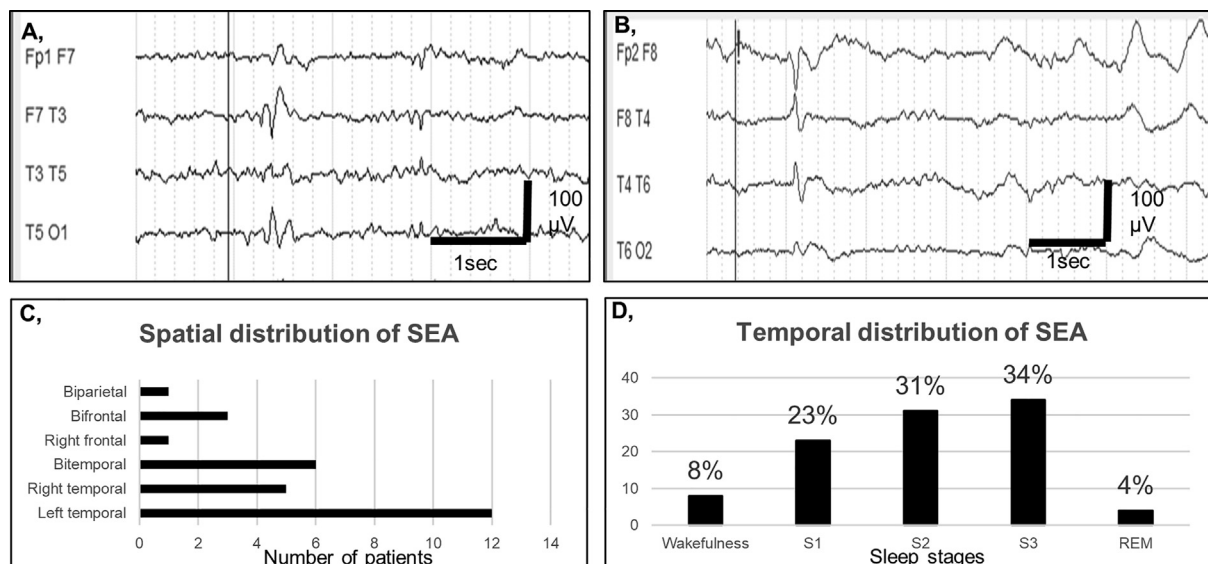
### 3.3. Characteristics of AD patients with and without subclinical epileptiform activity at year 0

Based on the presence or absence of SEA in the EEG recordings we divided our patients into two subgroups: AD + SEA (n: 28) and AD-SEA (n: 24) respectively. Presence of SEA was not associated with significantly different clinical or epidemiologic features of AD patients. They did not show differences in therapeutic regime, handedness, or in the course of dementia nor in global neuropsychology scores. Furthermore, patients with SEA showed higher (non-significant) VLOM ratios (Table 2). Analysis of various ACE subscores related to different cognitive domains revealed that patients with SEA had reduced performance in memory (Md:3.84;  $p:0.007$ ) and visuo-spatial scores (Md:1.05;  $p:0.03$ ) (Fig. 3). Difference in memory remained significant after Holm-Bonferroni correction ( $p < 0.008$ ).

### 3.4. Prospective analysis of the effect of subclinical epileptiform activity on the progression of Alzheimer’s disease at year 3

In our prospective study, we analyzed the data of 38 AD patients who completed the 3-year long follow-up (Table 3). AD + SEA patients (n = 21) differed only in the VLOM ratios from AD-SEA patients (n = 17) (Md:-0.57;  $p:0.039$ ). Spatial distribution of SEA at year 0 in the AD + SEA group was the following: left temporal (7/21 = 33%), right temporal (5/21 = 24%), bitemporal (4/21 = 19%), right frontal (1/21 = 5%), bifrontal (3/21 = 14%), biparietal (1/21 = 5%).

In the longitudinal analysis, AD + SEA patients showed significantly faster cognitive decline represented by average yearly decrease of total ACE scores (12.15 points/year in AD + SEA patients and 8.17 points/year in AD-SEA patients,  $F:15.891$ ;  $p:<0.001$ ) (Fig. 4) and by average yearly decrease in MMSE scores (2.71 points/year in AD + SEA patients and 2.22 points/year in AD-SEA patients,  $F:9.64$ ;  $p:0.01$ ). Cohen’s d effect size for 3-year ACE decline was 1.53 and 0.86 for 3-year MMSE decline. Significant differences were found with Tukey post-hoc analysis across the measured time points (1st, 2nd and 3rd years) in ACE and MMSE scores (all  $p$ 's < 0.001). The 1.5 times greater decrease in ACE and 20% greater decrease in MMSE in the presence of SEA remained significant in the covariance weighted analysis applying the onset of dementia (years), sex (% of females), educational level (total years) and 0 timepoint disease severity (MMSE score). In our cohort, only the 0 timepoint disease severity factor (MMSE score) showed significant progression-modifying effect ( $F:9.661$ ;  $p:<0.001$  for ACE;  $F:8.212$ ,  $p:0.01$  for MMSE). Decline in ACE score showed a significant positive correlation with the measured baseline spike frequency (year 0) ( $r:+0.664$ ;  $p:0.001$ ) (Fig. 5). We demonstrated smaller but significant correlation between spike frequency and reduction of MMSE score ( $r:+0.48$ ;  $p:<0.01$ ) as well. Since we found higher prevalence of spikes in the temporal regions with prominent left sided occurrence, we also measured the potential effect of spatial distribution of spikes on the progression of cognitive decline with ANOVA analysis comparing left, right and bitemporal appearances (N: 16). We found non-significant trend for differences ( $F:3.775$ ;  $p:0.051$ ) across the 3 groups, where left did not differ from bitemporal occurrence ( $p:1$ ), while right occurrence was associated with lower non-significant decrease in ACE than left appearance (Md: 11.85;  $p:0.058$ ) (Fig. 5).



**Fig. 2.** NEUROPHYSIOLOGIC FEATURES OF SUBCLINICAL EPILEPTIFORM ACTIVITY (SEA) IN AD PATIENTS AT YEAR 0. A: Spike detected in the left temporal region with phase inversion at temporal (T3) electrode in patient 005; B: Spike detected in the right frontotemporal region with maximum electronegativity at F8 electrode in patient 008; C: Spatial distribution of SEA showing the dominant occurrence of spikes in the temporal regions with left sided predisposition.; D: Temporal distribution of SEA demonstrating that spikes occur almost exclusively in sleep, dominantly in deep sleep.

**Table 2**  
Epidemiologic and clinical data of the AD + SEA and AD-SEA subgroups.

Parameter	AD-SEA	AD + SEA	p-value
Number of patients	24	28	-
Female sex (%)	14 (58%)	17 (61%)	0.579
Memantine therapy (%)	5 (20%)	6 (21%)	0.51
Cholinesterase inhibitor therapy (%)	24 (100%)	28 (100%)	1
Age (years, mean ± SD)	73.5 ± 7.8	71.9 ± 7.5	0.441
Right handedness (%)	21 (88%)	27 (96%)	0.321
Age at the onset of dementia (years, mean ± SD)	70.7 ± 7.5	69 ± 7.4	0.434
Duration of dementia in years with median (interquartile range)	3 (1–4)	3 (2–3)	0.76
Number of years of education in median (interquartile range)	12 (12–12)	12 (12–17)	0.26
MMSE median score (interquartile range)	19.5 (16–24.8)	20 (16–21.8)	0.665
ACE median score (interquartile range)	66 (58.5–69)	65 (55.3–77)	0.919
VLOM median ratio (interquartile range)	3.4 (3.2–3.6)	3.6 (3.3–4.6)	0.07

Statistical tests were Chi-square for sex, antidementia medication and handedness, t-test for age and for the onset of disease and Mann-Whitney U-test for MMSE score, ACE score and VLOM ratio.

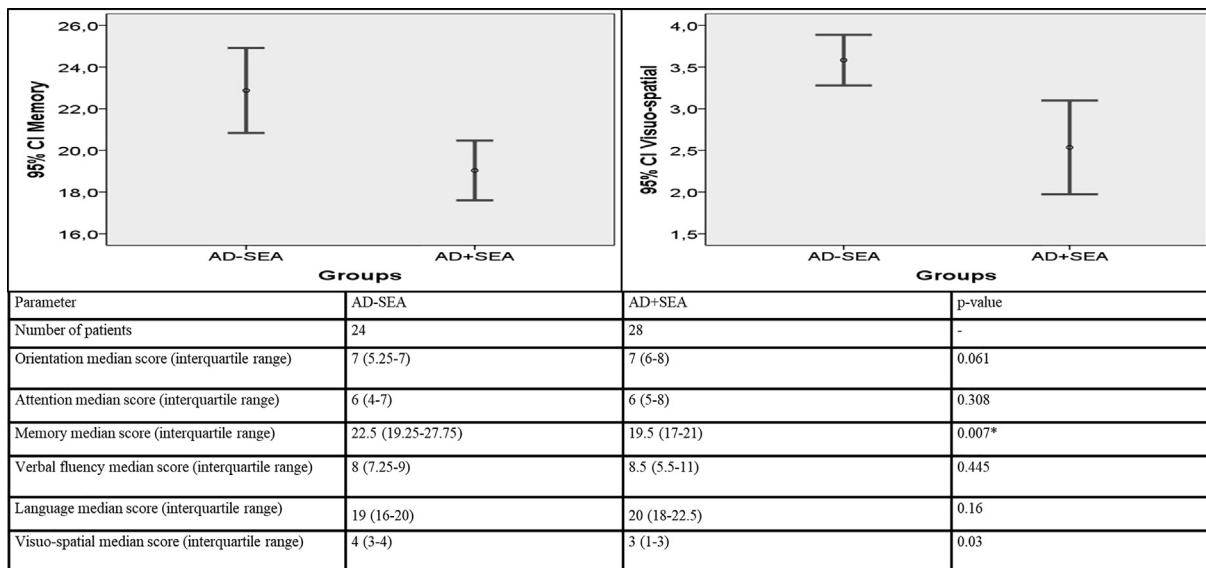
AD: Alzheimer’s disease; SEA: subclinical epileptiform activity; ACE: Addenbrooke Cognitive Examination; MMSE: Mini-Mental Score Examination; VLOM ratio: sum of verbal fluency and language scores divided by sum of orientation and delayed memory recall scores, SD: standard deviation.

**4. Discussion**

In the current study, we analyzed 24-hour EEGs of 52 AD patients and 20 healthy elderly controls. We demonstrated that AD patients show significantly higher prevalence of SEA than the control subjects (54% vs 20% respectively). Temporal distribution of SEA showed that 65% of the discharges occur in stage 2 or deeper sleep, while analysis of spatial occurrence revealed strong left side dominance. In the comparison of AD patients with and without SEA, we showed that patients with positive EEG had significantly lower performance in memory and visuo-spatial domains. In a 3-year prospective analysis of AD patients with repeated neuropsychological test battery, we revealed that patients with SEA showed significantly faster progression of cognitive decline.

There are only a few studies focusing on SEA in AD with ambiguous prevalence data. Liedorp and his colleagues found epileptiform discharges in only 2 % of 1674 AD patients (Liedorp et al., 2010). However, they performed only 30-min long daytime EEGs, therefore they could not detect most of the SEA events which, as we show in the present study, appear mostly during slow wave sleep. Also, Vossel et al found SEA in just 6% of 42 AD patients, that can be explained by evaluating only daytime routine EEGs in 91% of their patients (Vossel et al., 2013). Our remarkably higher – 54% - SEA rate may be due to the fact that we analyzed 24 hours EEGs including whole night sleep. It has been clearly shown in several epilepsy studies that interictal epileptiform discharges accumulate in sleep (Sammaritano et al., 1991; Steriade, 2003) and we had similar findings in AD patients in our previous study (Horvath et al., 2017) and in the current one as well (92% of spikes occurred in sleep). In the study of Vossel, the routine EEG was normal in 60% of AD patients who had manifest epileptic seizures, while sleep deprived serial EEGs showed epileptiform discharges only in 16% (Vossel et al., 2013). In our previous study we also showed that 1-hour sleep recording is twenty-times more sensitive in detecting epileptiform activity than 1-hour awake EEG (Horvath et al., 2017). Thus, the possible explanation for our finding of elevated SEA rate is the higher sensitivity of long-term EEG containing sleep recordings. This is supported by another study about the temporal distribution of SEA in AD showing that epileptic activity occurs almost exclusively (90%) during sleep (Vossel et al, 2016). Congruently with our data, these authors found SEA in 42% of AD patients.

In our healthy control sample, the relatively high incidence of SEA (25%) is curious; however, some studies also found increased risk for epileptiform event by non-epileptic elderly. In the study of McBride et al, epileptiform discharges were reported in 26% of 94 patients by patients aged 60 years and older using long-term EEG monitoring (McBride et al., 2002). Others found intermittent rhythmic delta activity in 17% of patients at the age 90 + applying 1-hour EEG recordings (Peltz et al., 2002). A study from Chochoi et al identified epileptiform discharges in 28% of 43 elderly patients



**Fig. 3.** DOMAIN SPECIFIC CHARACTERISTICS OF PATIENTS WITH (AD + SEA) AND WITHOUT SUBCLINICAL EPILEPTIFORM ACTIVITY (AD-SEA) AT YEAR 0. Mann-Whitney U-test was applied for pairwise comparisons. \* indicates significant differences ( $p < 0.008$ , after Holm-Bonferroni correction). AD: Alzheimer's disease; SEA: subclinical epileptiform activity.

**Table 3**

Baseline epidemiologic and clinical data of the AD-SEA and AD + SEA patient groups that completed the 3-year prospective follow-up.

Parameter	AD-SEA	AD + SEA	p-value
Number of patients	17	21	-
Female sex (n; %)	11 (65%)	11 (52%)	0.33
Memantine therapy (n; %)	4 (24%)	5 (24%)	0.94
Cholinesterase inhibitor therapy (n; %)	17 (100%)	21 (100%)	1
Age (years, mean ± SD)	74.2 ± 7.3	71.5 ± 5.9	0.22
Right handedness (%)	17 (100%)	21 (100%)	1
Age at the onset of dementia (years, mean ± SD)	70.8 ± 7.2	68.6 ± 5.5	0.304
Duration of dementia in years with median score (median; interquartile range)	3 (3–4)	3 (3–3)	0.055
Number of years of education in median score (interquartile range)	12 (12–14.5)	12 (12–17)	0.857
MMSE median score (interquartile range)	18 (15.5–22.5)	20 (16–21)	0.37
ACE score (mean ± SD)	65.5 ± 9.1	65.5 ± 12.5	0.98
VLOM median ratio (interquartile range)	3.3 (3.2–3.5)	3.6 (3.3–4.5)	*0.039

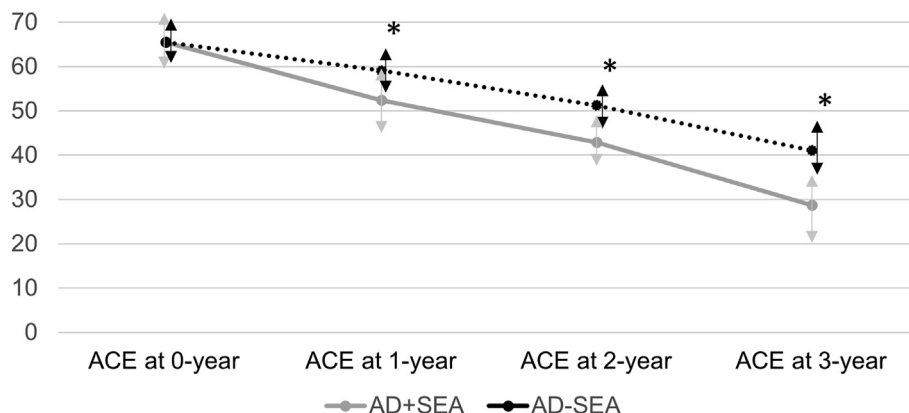
Statistical tests were Chi-square for sex, antedementia medication and handedness, t-test for age, for the onset of disease, for ACE score and Mann-Whitney U-test for MMSE score and VLOM ratio. \* indicates significant differences ( $p < 0.05$ ). AD: Alzheimer's disease; SEA: subclinical epileptiform activity; ACE: Addenbrooke Cognitive Examination; MMSE: Mini-Mental Score Examination; VLOM ratio: sum of verbal fluency and language scores divided by sum of orientation and delayed memory recall scores, SD: standard deviation.

using long-term EEG (Chochoi et al., 2017). A guideline report from Mayo Clinic proposes that SEA is presented in 12% of the healthy elderly (Davidson and Davidson, 2012). The incidence of SEA reaches 50% by non-epileptic elderly with syncope (Hughes and Zialcita, 2000). The significance of SEA in healthy elderly needs further studies in larger cohorts. As it has been reported by Vossel (Vossel et al., 2013) and was confirmed by our current findings too, SEA occurs in AD significantly more frequently than in healthy elderly. Interestingly, at the time of diagnosis (first neuropsychology testing) higher SEA did not associate with reduced global cognitive scores, however the more sensitive subscores of cognitive domains revealed associations to more severe impairment of

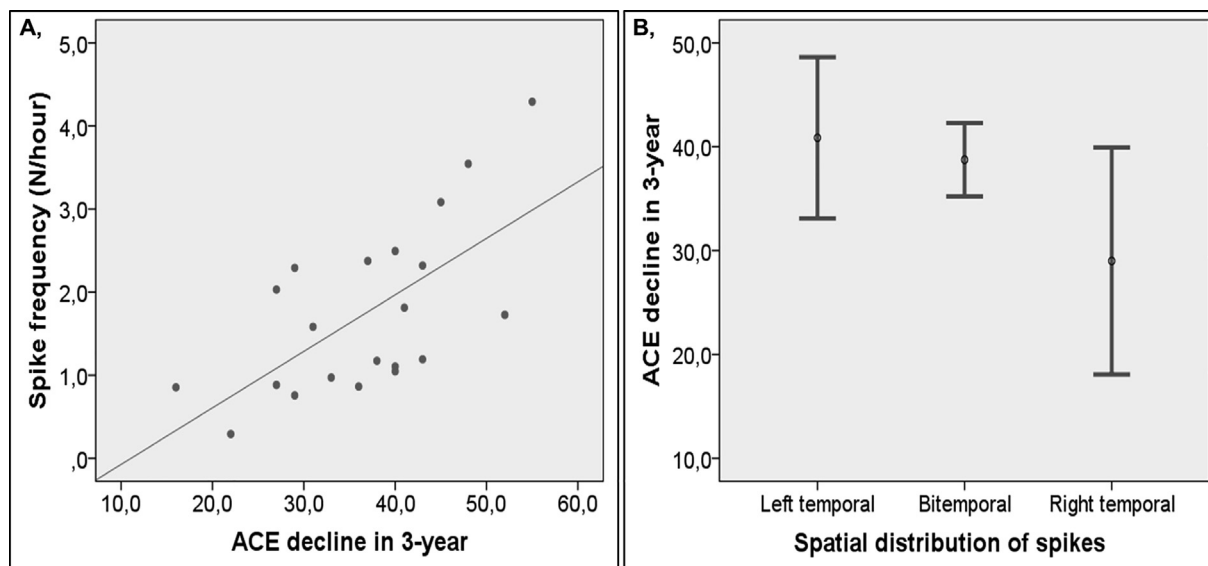
memory and visuo-spatial skills in our cohort. These findings suggest that SEA might serve as an indicator of faster progressing AD and become a more important contributor of disease progression in the later course of AD. These results need further investigations.

The spatial distribution of epileptiform discharges was scrutinized in only five studies; four of them analyzed AD patients with epileptic seizures (interictal epileptiform discharges) (Vossel et al., 2013; Cretin et al., 2016; Rao et al., 2009; Sarkis et al., 2016) and one of them investigated AD patients with SEA only (Vossel et al., 2016). In the summary of these reports, eighty percent of the discharges appeared in the frontotemporal regions, and 60% of them on the left side. In our study, SEA appeared almost exclusively in the temporal/fronto-temporal areas, involving the left side in 52%, the bitemporal areas in 26% and the right temporal electrodes in 22%. In view of the known temporal and frontal involvement of AD-related morphology changes, the frontotemporal dominance of the epileptiform activity is not surprising. However, the overwhelming left dominance of epileptiform activity in a neurodegenerative condition considered symmetric or leastwise bilateral, is curious. Noticeably, in frontotemporal dementia, a more severe left sided atrophy is a frequent imaging finding; nowadays we even consider this phenomenon a diagnostic hallmark (Frisoni et al., 1999). Interestingly, the strong left side dominance of interictal epileptiform discharges has been demonstrated in epilepsy studies as well (Doherty et al., 2003). If we accept that SEA or interictal activity are more frequent in the left temporal area and that higher spike frequency associate with faster cognitive decline, the clinical significance of this “left side phenomenon” warrants further investigations both in epilepsy and in neurodegenerative research.

The high prevalence of SEA is important if we consider the 1.5-times faster progression of cognitive deterioration of AD patients with the presence of SEA. The only available study in the literature focusing on the impact of SEA in AD had similar results (Vossel et al., 2016). In that study of Vossel et al, AD + SEA patients suffered 2.5-times faster decline in their yearly measured MMSE scores than patients with no SEA (Vossel et al., 2016). This finding remained significant even after correcting for the effects of age, gender and educational differences, similarly to our findings. In our cohort, we found smaller, 1.22-times faster decline in MMSE



**Fig. 4.** RESULTS OF LONGITUDINAL PROSPECTIVE FOLLOW-UP. AD + SEA patients show significant ( $p < 0.001$ ), 1.5-times higher decline in total ACE scores than AD-SEA patients using repeated general linear model. \* indicates significant differences ( $p < 0.001$ ). AD: Alzheimer's disease; SEA: subclinical epileptiform activity; ACE: Addenbrooke Cognitive Examination.



**Fig. 5.** RESULTS OF LONGITUDINAL PROSPECTIVE FOLLOW-UP AT YEAR-3 IN RELATION TO BASELINE (YEAR-0) FREQUENCY AND SPATIAL DISTRIBUTION OF EPILEPTIFORM SPIKES. A: the decline in ACE scores shows strong positive ( $r:+0.664$ ) and significant ( $p < 0.001$ ) correlation with spike frequency. B: left temporal ( $n:7$ ) and bitemporal ( $n:4$ ) spikes associate with faster cognitive decline than right spikes ( $n:5$ ) with marginal significance ( $p:0.051$ ). ACE: Addenbrooke Cognitive Examination.

scores in patients with SEA. The slower progression rate in MMSE scores might be explained by the differences among the subjects of the two study samples in both the AD + SEA and AD-SEA groups (2.71 points/year in AD + SEA patients and 2.22 points/year in AD-SEA patients in our report vs 3.9 points/year in patients with epileptiform activity vs. 1.6 points/year in patients without in Vossel's experiment). In our cohort, we analyzed older patients (average age for prospective study was 73 in our cohort and ~ 61 in Vossel's study), with shorter duration of dementia (~3 years in our study vs ~ 5 years in Vossel's experiment) and with higher proportion of patients taking antedementia medication (24% for Memantine and 100% for cholinesterase inhibitor therapy in our study vs 2.5% for Memantine and 56% for cholinesterase inhibitor therapy in Vossel's report). All of these factors might influence the progression of AD. However, we could replicate the major striking results with a more sensitive neuropsychological test battery (ACE versus MMSE) and larger sample size (38 vs 26 patients). We also revealed a direct link between spike activity and cognitive decline with the demonstration of strong correlation across spike frequency and decline in cognitive scores.

There are limitations to our study. Firstly, we did not perform EEG examination at the end of the follow-up, so there is a possibility that spike frequency and spatial distribution have changed during the follow-up period. Another important limitation is that positron emission tomography, cerebrospinal fluid analysis or genetic testing were not applied in the current experiment. Furthermore, AD group was significantly older than the control group ( $75.5 \pm 8$  vs  $67.8 \pm 4.8$  years respectively), however, statistical analysis was corrected for age. The strengths of the current report are the rigorous patient selection, the long follow-up period, and the application and analysis of long-term EEG containing sleep.

To conclude, 24-hour EEG recording detects subclinical epileptiform activity in more than half of the AD patients. SEA appears typically in the frontotemporal areas, predominantly in the left hemisphere and it seems to accelerate the progression of AD. Based on our findings it seems reasonable that development of new antiepileptic drugs targeting SEA control might represent a promising a novel strategy to slow cognitive decline in Alzheimer's disease. More studies are required to further elucidate the importance of epileptiform activity in neurodegenerative disorders.

## Funding

Our study was supported by National Brain Research Program I, II (KTIA\_NAP\_13-1-2013-0001; 2017-1.2.1-NKP-2017-00002), Hungarian Scientific Research Fund 2019 of the National Research, Development and Innovation Office (PD- 132652), Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences (bo\_78\_20\_2020). This is an EU Joint Programme- Neurodegenerative Disease Research (JPND) project. The project is supported through the following funding organization under the aegis of JPND- www.jpnd.eu (National Research, Development and Innovation, Hungary, 2019-2.1.7-ERA-NET-2020-00006).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We thank all the patients who participated in this study. Special thanks to Zita Csepella for analyzing the EEG recordings.

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