Analysis of MEG Background Activity in Alzheimer's Disease Using Nonlinear Methods and ANFIS

Analysis of the MEG background activity in Alzheimer's disease using non-linear methods and ANFIS

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Abstract–The aim of the present study was to analyze the magnetoencephalogram (MEG) background activity from 20 patients with probable Alzheimer's disease (AD) and 21 control subjects using two non-linear methods: sample entropy (*SampEn*) and Lempel-Ziv complexity (*LZC*). The former quantifies the signal regularity, while the latter is a complexity measure. The signals were acquired with a 148-channel whole-head magnetometer placed in a magnetically shielded room. Our results show that MEG recordings are less complex and more regular in AD patients than in control subjects. Significant differences between both groups were found in 16 MEG channels with *SampEn* and in 134 with *LZC* ($p < 0.01$, Student's *t*-test with Bonferroni's correction). Using receiver operating characteristic curves with a leave-one-out cross-validation procedure, accuracies of 70.73% and 78.05% were reached with *SampEn* and *LZC*, respectively. Additionally, we wanted to assess whether both non-linear methods and an adaptive-network-based fuzzy interference system (ANFIS) could improve AD diagnosis. With this classifier, an accuracy of 85.37% was achieved. Our findings suggest the usefulness of our methodology to increase our insight into AD.

Keywords–Adaptive-network-based fuzzy interference system (ANFIS), Alzheimer's disease, Lempel-Ziv complexity, magnetoencephalogram, sample entropy

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive and irreversible brain disorder of unknown aetiology. It affects 1% of the population aged 60-64 years, but the prevalence increases exponentially with age, so around 30% of people over 85 years suffer from this disease.[24] Additionally, due to the fact that life expectancy has increased significantly in western countries during the last decades, it is expected that the number of people with dementia will increase to 81 million in 2040.[24] Clinically, this degenerative neurological disease manifests itself as a slowly progressive impairment of mental functions whose course lasts several years prior to death. AD patients may wander, be unable to engage in conversation, appear non-responsive, become helpless and need complete care and attention.[7,22] The clinical characteristics at the microscopic level include senile plaques containing amyloid-beta-peptide and neurofibrillary tangles in the medial temporal lobe structures and cortical areas of the brain.[6] AD is also characterized by loss of neurons and synapses.

The criteria of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA)[29] are commonly used for the clinical diagnosis of AD. According to NINCDS–ADRDA, AD can be classified as definite (clinical diagnosis with histologic confirmation), probable (typical clinical syndrome without necropsy confirmation) or possible (atypical clinical features but no alternative diagnosis apparent).[7] In order to reduce the damage suffered by the patient's brain and to adopt more efficient drug taking strategies, an early diagnosis is needed. The differential diagnosis with other types of dementia and with major depression includes medical history, physical and neurological evaluation, laboratory studies and neuroimaging techniques. Mental status tests are also used to assess the severity of cognitive deficit. However, a definite diagnosis is only possible by necropsy. Hence, new approaches are needed to improve AD detection.

Nowadays, electroencephalography (EEG) and magnetoencephalography (MEG) recordings are not used in AD clinical diagnosis. Nevertheless, several studies have demonstrated that the

analysis of EEG/MEG signals could help physicians in the diagnosis of this dementia (extensive reviews can be found in Jeong[22] and Stam[39]). Both EEG and MEG are non-invasive techniques that allow to record the electromagnetic fields produced by brain activity with good temporal resolution. The use of MEG recordings to study the background brain activity offers some advantages over EEG. MEG provides reference-free recordings, which are not distorted by the resistive properties of the skull.[14] Additionally, MEG offers higher spatial resolution than conventional EEG.[14] On the other hand, the magnetic signals generated by the human brain are extremely weak. Thus, SQUID (Superconducting QUantum Interference Device) sensors are necessary to detect them and MEGs must be recorded in a magnetically shielded room. Therefore, MEG is characterized by limited availability and high equipment cost.

Until the introduction of methods derived from non-linear dynamics, AD patients' brain recordings were analyzed visually or with linear techniques based on coherence and spectral calculations.[22] These analyses seem to discriminate AD patients from control subjects through an increased EEG/MEG activity in lower frequency bands associated with AD.[10,37] On the other hand, non-linear methods have also demonstrated their usefulness in the analysis of the EEG/MEG background activity in AD.[22,39] The first non-linear methods used to study the brain recordings from AD patients were correlation dimension ($D2$) and the first Lyapunov exponent ($L1$). Jeong *et al.*[20] showed that AD patients exhibit significantly lower $D2$ and $L1$ values than controls in most EEG channels. Using $D2$, another study revealed a decreased complexity of the MEG background activity in AD patients in the low frequency bands, and an increase in the high bands.[40] However, these classical measures for complexity estimation have some drawbacks. Reliable estimation of $L1$ and $D2$ requires a large number of data points and stationary and noise-free time series.[8,20] These requirements are difficult to fulfill for physiological data. Hence, other non-linear methods are necessary to study brain recordings. For instance, Abásolo *et al.*[2] found significant differences in some EEG channels with sample entropy (*SampEn*), concluding that the EEG background activity is more regular in AD patients than in control subjects. EEG/MEG studies demonstrated that AD patients have significantly

lower Lempel-Ziv complexity (*LZC*) values than elderly control subjects.[3,11]

The application of neural networks and fuzzy logic techniques to classify AD patients' brain recordings has not received much attention. Besthorn *et al.*[5] employed a neural network to recognize the EEGs from AD patients and controls. Petrosian *et al.*[32] reached a sensitivity of 80% at 100% specificity using a recurrent multi-layer perceptron. In the current work, the classification task is performed by an adaptive-network-based fuzzy interference system (ANFIS).[36] ANFIS combines the adaptive capabilities of neural networks and the qualitative approach of fuzzy logic.[13] Moreover, it has already been successfully applied for the classification of biological time series, such as EEG[13] or electromyographic recordings.[18]

In this study, we have examined the MEG background activity in 20 patients with probable AD and 21 control subjects with two non-linear methods: *SampEn* and *LZC*. The former quantifies the signal regularity, while the latter is a complexity measure. Thus, *SampEn* and *LZC* could provide complementary information to improve the AD diagnosis. Our goal was to test the hypothesis that AD patients' recordings are more regular and less complex than controls' MEGs, indicating the presence of abnormal brain dynamics associated with AD. Furthermore, we wanted to asses whether the use of an ANFIS classifier yields a higher diagnostic accuracy than the sole non-linear methods.

2. MATERIALS AND METHODS

2.1. Subjects

In the present study, MEG signals were recorded from 41 subjects. All patients and controls underwent an exhaustive neuropsychological evaluation including the Spanish versions of the following scales and batteries: Wechsler Memory Scale 3rd Edition (WMS-III), Boston Naming Test (BNT), Stroop Test, Wisconsin Card Sorting Test (WCST), Silhouettes Test of the Visual Object and Space Battery (VOSP), and tests for constructive and ideatory apraxia. Cognitive status was screened in both groups with Mini Mental State Examination (MMSE).

MEGs were obtained from twenty patients (7 men and 13 women; age = 73.05 ± 8.65 years,

mean ± standard deviation, SD) fulfilling the criteria of probable AD. They were recruited from the "Asociación de Familiares de Enfermos de Alzheimer" in Spain. Diagnosis for all patients was made according to the NINCDS–ADRDA criteria.[29] The mean MMSE score for these patients was 17.85 ± 3.91 (mean ± SD). Patients were free of significant medical, neurological and psychiatric diseases other than AD and they were not taking drugs which could affect MEG activity.

The control group consisted of 21 elderly control subjects without past or present neurological disorders (9 men and 12 women; age = 70.29 ± 7.07 years, MMSE score = 29.10 ± 1.00 points, mean ± SD). The difference in age between both populations was not statistically significant (p-value = 0.2752, Student's t-test). All control subjects and patients' caregivers signed an informed consent for the participation in this research work. The local Ethics Committee approved this study.

2.2. Magnetoencephalogram recordings

MEGs were acquired with a 148-channel whole-head magnetometer (MAGNES 2500 WH, 4D Neuroimaging) placed in a magnetically shielded room at "Centro de Magnetoencefalografía Dr. Pérez-Modrego" (Spain). The subjects lay on a patient bed, in a relaxed state and with their eyes closed. For each subject, five minutes of recording were acquired at a sampling frequency of 678.17 Hz, using a hardware band-pass filter from 0.1 to 200 Hz. Then, the equipment decimated each 5 minute data set. This process consisted of filtering the data to satisfy the Nyquist criterion, following by a down-sampling by a factor of 4, thus obtaining a sampling rate of 169.549 Hz. Finally, artifact-free epochs of 10 seconds were processed using a band-pass filter with a Hamming window and cut-off frequencies at 0.5 and 40 Hz.

2.3. Methods

MEG epochs were analyzed by means of two non-linear methods: *SampEn* and *LZC*. Afterward, statistical analyses were used to determine if there were any differences between the

values obtained in both groups: AD patients and elderly control subjects. Finally, the results of both non-linear methods were used as input to an ANFIS classifier. In fig. 1, we present the steps followed in this study.

DISPLAY FIGURE 1 AROUND HERE

2.3.1. Sample entropy (*SampEn*)

SampEn is an embedding entropy that quantifies the signal irregularity: more irregularity in the data produces larger *SampEn* values.[35] *SampEn* is the negative natural logarithm of the conditional probability that two sequences similar for m points remain similar at the next point.[35] This metric solves some problems associated with approximate entropy (*ApEn*), a non-linear method introduced by Pincus[33] to quantify the regularity of time series, initially motivated by applications to relatively short, noisy data sets. *SampEn* is largely independent of the signal length and displays relative consistency under circumstances where *ApEn* does not. Additionally, the algorithm used to compute the *SampEn* is simpler than the *ApEn* algorithm.[35]

To calculate *SampEn*, two input parameters must be specified: a run length m and a tolerance window r. The values of m and r are critical in the performance of *SampEn* and comparisons between time series can be done only with fixed values of m, r and N, where N is the number of samples in the time series. In order to avoid a significant contribution of noise in the *SampEn* estimation, r must be higher than most of the noise.[33] Additionally, if r is too small, the entropy estimation might fail.[9] In addition to this, the accuracy and confidence of the *SampEn* estimate improve for low m values and large r values, since the number of matches of length m and $m + 1$ increases.[27] The existing rules lead to the use of r values between 0.1 and 0.25 times the standard deviation of the original time series and m values of 1 or 2, for signals from 100 to 5000 data points.[27] In our study, we have chosen $m = 1$ and $r = 0.25$ times the standard deviation of the original time series. These values follow the aforementioned guidelines and have been used in a previous AD study.[2] This measure has already been used to study some biological

signals, such as heart rate time series[27] and EEG data.[2]

Given a one dimensional time series $X = x(1), x(2),..., x(N)$, the algorithm to compute the *SampEn* can be described as:[35]

- Form $N - m + 1$ vectors $X_m(i)$ defined by: $X_m(i) = x(i), x(i + 1),..., x(i + m - 1)$, with $1 \le i \le N - m + 1$.
- The distance between two of these vectors, $X_m(i)$ and $X_m(j)$, is the maximum absolute difference between their respective scalar components:

$$d[X_m(i), X_m(j)] = \max(|x(i+k) - x(j+k)|), \quad (1)$$

for $0 \le k \le m - 1$.

- Define $B_i^m(r)$ as $1/(N - m - 1)$ times the number of vectors $X_m(j)$ within r of $X_m(i)$, where $1 \le j \le N - m$, $(j \ne i)$. Then, set $B_m(r)$ as:

$$B_m(r) = \frac{1}{N-m} \sum_{i=1}^{N-m} B_i^m(r). \quad (2)$$

- Similarly, calculate $A_i^m(r)$ as $1/(N - m - 1)$ times the number of j $(1 \le j \le N - m; j \ne i)$, such that the distance between $X_{m+1}(j)$ and $X_{m+1}(i)$ is less than or equal to r. Set $A_m(r)$ as:

$$A_m(r) = \frac{1}{N-m} \sum_{i=1}^{N-m} A_i^m(r). \quad (3)$$

- Finally, define:

$$SampEn(m, r) = \lim_{N \to \infty} \left[-\ln \frac{A_m(r)}{B_m(r)} \right], \quad (4)$$

which is estimated by the statistic

$$SampEn(m, r, N) = -\ln \frac{A_m(r)}{B_m(r)}. \quad (5)$$

2.3.2. Lempel-Ziv complexity (*LZC*)

The *LZC* algorithm was proposed by Lempel and Ziv to evaluate the randomness of finite sequences.[28] It is a nonparametric and simple-to-compute measure of complexity for one-

dimensional signals that does not require long data segments to be calculated.[41] Larger *LZC* values correspond to more complex data. *LZC* has been widely applied to EEG/MEG data and other biomedical signals.[3,11, 31,41]

LZC analysis is based on a coarse-graining of the measurements, so the MEG time series must be transformed into a finite symbol sequence. In this study, we used the simplest way: a binary sequence conversion (zeros and ones), since previous studies suggested that this kind of conversion may keep enough signal information.[41] The median value is used as the threshold T_d, due to the fact that partitioning about the median is robust to outliers.[31] By comparison with T_d, the original data are converted into a 0-1 sequence $P = s(1), s(2),\ldots, s(N)$, with $s(i)$ defined by:[41]

$$s(i) = \begin{cases} 0 & if\ x(i) < T_d \\ 1 & if\ x(i) \geq T_d \end{cases}. \tag{6}$$

The string P is scanned from left to right and a complexity counter $c(N)$ is increased by one unit every time a new subsequence of consecutive characters is encountered in the scanning process. The detailed algorithm for the measure of *LZC* is as follows:[41]

- Let S and Q denote two subsequences of the original sequence P. SQ is the concatenation of S and Q, while $SQ\pi$ is a string derived from SQ after its last character is deleted (π means the operation to delete the last character). Let $v(SQ\pi)$ denote the vocabulary of all different substrings of $SQ\pi$.
- At the beginning, the complexity counter $c(n) = 1$, $S = s(1)$, $Q = s(2)$, $SQ = s(1), s(2)$ and $SQ\pi = s(1)$.
- For generalization, suppose that $S = s(1), s(2),\ldots, s(r)$, $Q = s(r+1)$ and, therefore, $SQ\pi = s(1), s(2),\ldots, s(r)$. If $Q \in v(SQ\pi)$, then Q is a subsequence of $SQ\pi$, not a new sequence.
- S does not change and renew Q to be $s(r+1), s(r+2)$, then judge if Q belongs to $v(SQ\pi)$ or not.
- The previous steps are repeated until Q does not belong to $v(SQ\pi)$. Now $Q = s(r+1), s(r+2),\ldots, s(r+i)$ is not a subsequence of $SQ\pi = s(1), s(2),\ldots, s(r+i-1)$, so increase the

counter by one.

- Thereafter, S and Q are combined and renewed to be $s(1)$, $s(2)$,…, $s(r+i)$, and $s(r+i+1)$, respectively.
- Repeat the previous steps until Q is the last character. At this time, the number of different substrings is $c(N)$, the measure of complexity.

In order to obtain a complexity measure independent of the sequence length, $c(N)$ should be normalized. If the length of the sequence is N and α is the number of different symbols, it has been proved that the upper bound of $c(N)$ is given by:[28]

$$c(N) < \frac{N}{(1-\varepsilon_N)\log_\alpha(N)}, \tag{7}$$

where ε_N is a small quantity and $\varepsilon_N \to 0$ ($N \to \infty$). In general, $N/\log_\alpha(N)$ is the upper limit of $c(N)$, i.e.,

$$\lim_{N\to\infty} c(N) = b(N) \equiv \frac{N}{\log_\alpha(N)}. \tag{8}$$

For a binary conversion $\alpha = 2$, $b(N) \equiv N/\log_2(N)$ and $c(N)$ can be normalized via $b(N)$:

$$C(N) = \frac{c(N)}{b(N)}. \tag{9}$$

The normalized *LZC* reflects the arising rate of new patterns along with the sequence.

2.3.3. Adaptive-Network-Based Fuzzy Interference System (ANFIS)

ANFIS is an adaptive network originally described by Roger Jang.[36] It is functionally equivalent to a fuzzy interference system consisting of a rules set. ANFIS architecture consists of five layers: fuzzy layer, product layer, normalized layer, defuzzy layer and total output layer. The entire system architecture chosen for this study is shown in fig. 2 and is described below (Note that O_i^j denotes the output of the i-th node in the j-th layer):[36]

- The first layer contains three adaptive nodes for each input,[16,25] with node functions:

$$O_i^1 = \begin{cases} \mu_{A_i}(x) & i = 1,2,3 \\ \mu_{B_{i-3}}(y) & i = 4,5,6 \end{cases}. \tag{10}$$

where x (or y) is the input to node i, and A_i (or B_i) are the membership functions. These functions map the input x (or y) into the output μ_{A_i} (or μ_{B_i}). As membership function, Roger Jang[36] suggests the use of a Gaussian bell-shaped, with maximum equal to 1 and minimum equal to 0:

$$\mu_{A_i}(x) = \frac{1}{1 + \left[\left(\frac{x - c_i}{a_i}\right)^2\right]^{b_i}}, \tag{11}$$

where a_i, b_i and c_i are called premise parameters.

- In the second layer, every node is identified as *M*. They are fixed nodes whose outputs are the product of the incoming signals:

$$O_i^2 = \omega_i = \mu_{A_m}(x) \times \mu_{B_n}(y), \tag{12}$$

for i = 1, 2, ..., 9, m = 1, 2, 3, and n = 1, 2, 3. The output of each node represents the firing strength of a rule.

- Layer 3 contains nodes labeled as *N*. The *i*-th node calculates the ratio of the *i*-th rule firing strength to the sum of all firing strengths:

$$O_i^3 = \overline{\omega}_i = \frac{\omega_i}{\omega_1 + \omega_2 + ... + \omega_9}. \tag{13}$$

The outputs of this layer are called normalized firing strengths.

- Layer 4 is formed by adaptive nodes with node functions:

$$O_i^4 = \overline{\omega}_i f_i = \overline{\omega}_i (p_i x + q_i y + r_i), \tag{14}$$

where $\overline{\omega}_i$ is the output of the *i*-th node of layer 3 and p_i, q_i and r_i is a parameter set. These parameters are termed consequent parameters.

- The single node of layer 5 computes the overall output as the summation of all incoming signals:

$$O_1^5 = \sum_{i=1}^{9} \varpi_i f_i \ . \tag{15}$$

On the contrary to a conventional fuzzy interference system, the parameters of the membership functions are not determined manually by an expert, but automatically based on a training set of input/output data.[16] ANFIS were trained with a hybrid learning algorithm that combines the gradient descent method and the least squares method to identify the parameter sets of layers 1 and 4.[36]

In our study, a leave-one-out procedure was used to asses the classification performance of ANFIS. In this procedure, the mean *SampEn* and *LZC* values obtained from one subject's epochs are left out, and the non-linear results from the remaining subjects' epochs are used as training data. ANFIS learns features in this data set and adjusts automatically the parameter sets according to a given error criterion.[36] The left-out subject is then classified by this trained network. This procedure is repeated for all subjects.

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3. RESULTS

The *SampEn* algorithm was applied to all 148 MEG channels with $m = 1$ and $r = 0.25$ times the SD of the original time series. The average *SampEn* value for the control group was 1.24 ± 0.14 (mean ± SD), whereas it reached 0.97 ± 0.26 for the AD patients. Our results showed that *SampEn* values were higher in the control subjects than in the AD patients' group for all channels, which suggests that AD is accompanied by a MEG irregularity decrease. Moreover, we calculated the *p*-values of the Student's *t*-test with Bonferroni's correction to determine whether there were significant differences between both groups. Statistically significant differences ($p < 0.01$) were found in 16 channels.

We also computed the *LZC* and calculated the *p*-values of the Student's *t*-test (Bonferroni's correction) for each MEG channel. AD patients had lower *LZC* values than control subjects at all MEG channels. Average *LZC* values were 0.69 ± 0.04 for the control group and 0.57 ± 0.08

in AD patients. These results show MEG background activity of AD patients is less complex than in a normal brain. Moreover, the differences between AD patients and elderly control subjects were statistically significant in 134 channels ($p < 0.01$, Student's *t*-test with Bonferroni's correction).

Additionally, ROC curves were used to assess the ability of *SampEn* and *LZC* to discriminate AD patients from control subjects. This statistical method summarizes the performance of a two-class classifier across the range of possible thresholds. It is a graphical representation of the trade-offs between sensitivity and specificity. Sensitivity is the true positive rate while specificity is equal to the true negative rate. Accuracy is the percentage of subjects (AD patients and controls) correctly recognized. A leave-one-out cross-validation procedure was used to calculate sensitivity, specificity, and accuracy values. In the leave-one-out method, the data from one subject are excluded from the training set one at a time and then classified on the basis of the threshold calculated from the data of all other subjects. The leave-one-out cross-validation procedure provides a nearly unbiased estimate of the true error rate of the classification procedure.[38] Mean values, obtained averaging the results of all channels, were used to plot the ROC curves shown in Fig. 3. With *SampEn* results, a sensitivity of 80% and a specificity of 61.9% were achieved. The results were better when the mean *LZC* values were analyzed: an accuracy of 78.05% was reached. Sensitivity, specificity, and accuracy values for each non-linear measure (*SampEn* and *LZC*) are shown in Table 1.

DISPLAY FIGURE 3 AROUND HERE

Finally, the results of both non-linear methods were used as the inputs to the ANFIS classifier shown in Fig. 2. A leave-one-out procedure was used to asses the classification performance of ANFIS. An accuracy of 85.37% (85.0%, sensitivity; 85.71% specificity) was achieved. An increase of 7.32% in the accuracy with respect to the results obtained using only the *LZC* was reached, as can be noticed in Table 1.

INSERT TABLE 1 AROUND HERE

4. DISCUSSION AND CONCLUSIONS

We analyzed the MEG background activity from 20 patients with probable AD and 21 elderly control subjects by means of two non-linear methods: *SampEn* and *LZC*. Our purpose was to check the hypothesis that MEG background activity is different in AD patients and control subjects.

SampEn has proven to be effective in discriminating AD patients from controls subjects. Our study revealed that AD patients have lower *SampEn* values than controls at all channels. These results are in agreement with previous research works that have applied non-linear methods to estimate the regularity of the AD patients' brain activity.[1,2,12,17] *ApEn* values were significantly lower in the EEG of AD patients at electrodes P3 and P4,[1] whereas statistically significant differences were found at P3, P4, O1 and O2 using *SampEn*.[2]

Our results also showed that AD patients have lower *LZC* values than controls. Moreover, significant statistical differences were found in most MEG channels. These results agree with other studies that showed a decreased complexity in the brain recordings from AD patients. For instance, Escudero *et al.*[9] found significant differences in some EEG channels with multiscale entropy. Other EEG/MEG studies demonstrated that AD patients had lower *LZC* values than controls.[3,11] Despite their drawbacks, traditional non-linear methods, like $D2$ and $L1$, also have been used to estimate the complexity of EEG/MEG recordings.[5,20,40] Previous studies have suggested that $D2$ and $L1$ values are lower in AD patients' EEGs than in controls' ones.[20] Besides, significant differences between AD patients and control subjects were found in almost all EEG channels.[20] Van Cappellen van Walsum *et al.*[40] estimated $D2$ in different MEG frequency bands, finding statistical differences between AD patients and age-matched controls in delta, theta and beta bands.

Our findings support the notion that AD involves an overall loss of irregularity and complexity in the electromagnetic brain activity. Although this complexity/irregularity reduction seems to be associated with the deficiencies in information processing suffered by AD

patients, its pathophysiological implications are not clear. It might be due to neuronal death, a loss of synaptic connections, a general effect of neurotransmitter deficiency or a loss of dynamical brain responsivity to stimuli.[19,20] Although a loss of physiological complexity and irregularity often accompanies ageing,[26] in the present study the groups were matched for age. Furthermore, the significantly reduced complexity/irregularity may represent the cognitive dysfunction in AD.

ROC curves with a leave-one-out cross-validation procedure were used to assess the ability of *SampEn* and *LZC* to classify AD patients and control subjects. Using *SampEn*, an accuracy of 70.73% (80%, sensitivity; 61.9% specificity) was achieved. With *LZC*, specificity of 76.19%, sensitivity of 80%, and accuracy of 78.05% were reached. In previous papers, spectral parameters and non-linear methods have been used to distinguish AD patients and control subjects. The accuracy values achieved in the aforementioned studies are shown in Table 2. Nevertheless, all these values should be taken with caution due to the small sample sizes. Moreover, it is noteworthy that a leave-one-out cross-validation procedure has been used in our study and in Hornero *et al.*[17], but not in the other ones. Despite the fact that the accuracy decreases with this procedure, it provides a nearly unbiased estimate of the true error rate of the classification method.[38]

INSERT TABLE 2 AROUND HERE

SampEn and *LZC* values were used as input to an ANFIS classifier with a leave-one-out cross-validation procedure. An accuracy of 85.37% was achieved with this adaptive network. In order to demonstrate the usefulness of ANFIS in differentiating AD patients from controls, this value was compared with the accuracies obtained using the non-linear methods described in previous AD studies: auto-mutual information,[12] spectral entropy,[2,17] *ApEn*,[1,17] *SampEn*[2] and *LZC*.[3,11] These methods were applied to the same MEG database of the current study and a leave-one-out cross-validation procedure was used. The accuracy values reached were: 73.17% with auto-mutual information, 73.17% when spectral entropy was used, 60.98% with *ApEn*,

70.73% using *SampEn* and, finally, 78.05% with *LZC*. These values show that the use of an ANFIS classifier, together with *SampEn* and *LZC*, may be more useful in detection of AD than the methodologies based on a single parameter. To the best of our knowledge, there are no papers available on AD diagnosis using non-linear methods and ANFIS. Therefore, we have presented a new technique that might be useful in the diagnosis of this dementia.

Our results show that *SampEn* and *LZC* are adequate methods to differentiate the MEG activity from AD patients and control subjects. Nevertheless, some limitations of our study merit consideration. Firstly, the sample size is small to obtain decisive results. Moreover, the detected decrease in irregularity and complexity is not specific to AD. It appears in other brain disorders, like epilepsy,[23] schizophrenia[30] or vascular dementia.[21] Future efforts will be addressed to explore other non-linear measures to characterize MEG background activity in AD and in other pathologies. It is particularly interesting to study MEGs from patients with mild cognitive impairment, since several authors have considered this disease as a prodromal phase of AD.[22] Furthermore, our results do not show if *SampEn* and *LZC* can detect a gradation of the disease process. Finally, the results obtained from each parameter were averaged to simplify the analyses. This issue involves a loss of spatial information, which could be partially avoided by computing the mean of each parameter for a number of brain regions.

In conclusion, non-linear analysis of the MEG background activity with *SampEn* and *LZC* revealed an increased regularity and a decreased complexity of the AD patients' MEGs. Our results suggest that neuronal dysfunction in AD is associated with differences in the MEG background activity. Additionally, we have demonstrated the usefulness of an ANFIS classifier in order to improve AD diagnosis.

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TABLE CAPTIONS

Table 1. Sensitivity, specificity, and accuracy values obtained with *SampEn*, *LZC* and ANFIS, using a leave-one-out cross-validation procedure.

Table 2. Summary of articles concerning the classification of AD patients versus control subjects. The highest accuracy values reached in each paper are shown.

TABLE 1

	Sensitivity	Specificity	Accuracy
SampEn	80.00%	61.90%	70.73%
LZC	80.00%	76.19%	78.05%
ANFIS	85.00%	85.71%	85.37%

TABLE 2

Paper	Data set	Method	Highest accuracy values (%)
Abásolo *et al.* 2005[1]	10 AD patients and 8 controls (EEG)	Approximate entropy	83.3% (ROC curve at electrode P3)
Abásolo *et al.* 2006[2]	11 AD patients and 11 controls (EEG)	Sample entropy	72.3% (ROC curve at EEG electrodes P3, P4, O1 and O2)
Abásolo *et al.* 2006[3]	11 patients with AD and 11 control subjects (EEG)	Lempel-Ziv complexity	81.8% (ROC curve at P3 and O1 with a two-symbol sequence conversion, and at P3, P4 and O1 with a three-symbol conversion)
Bennys *et al.* 2001[4]	35 patients with AD and 35 controls (EEG)	Spectral ratios	82.8% (Ratio theta/(alpha+beta1) at the left temporal cerebral region analyzed with a ROC curve)
Besthorn *et al.* 1997[5]	50 AD patients and 42 control subjects (EEG)	Correlation dimension	69.5% (Neural network)
Escudero *et al.* 2006[9]	11 AD patients and 11 control subjects (EEG)	Multiscale entropy	90.9% (ROC curve at EEG electrode Fp1)
Gómez *et al.* 2006[11]	21 patients with AD and 21 elderly controls (MEG)	Lempel-Ziv complexity	83.3% (First principal score from principal component analysis examined with a ROC curve)
Gómez *et al.* 2007[12]	20 AD patients and 21 controls (MEG)	Auto mutual information	82.9% (Mean values analyzed with a ROC curve)
Henderson *et al.* 2006 [15]	17 patients with probable AD and 24 control subjects (EEG)	Fractal dimension and cumulative density of zero-crossing intervals	Sensitivities of 67% (fractal dimension) and 78.8% (zero-crossing method) with a specificity fixed to 99.9%
Hornero *at al.* 2008[17]	20 patients with AD and 21 elderly controls (MEG)	Spectral and non-linear methods	80.5% with a linear discriminant analysis (median frequency and *ApEn*)
Petrosian *et al.* 2001[32]	10 AD patients and 10 control subjects (EEG)	Wavelets	85.7% (Three layer recurrent neural network)
Poza *et al.* 2007[34]	20 patients with AD and 21 controls (MEG)	Five spectral parameters	85.4% (First principal component from mean frequency values analyzed with a ROC curve)
Current study	20 patients with AD and 21 controls (MEG)	Sample entropy, Lempel-Ziv complexity and ANFIS	70.7% (Mean *SampEn* values and a ROC curve) 78.0% (Mean *LZC* values and a ROC curve) 85.4% (ANFIS classifier)

FIGURE LEGENDS

Figure 1. Block diagram of the steps followed in the MEG analysis: signal pre-processing, regularity and complexity analysis with *SampEn* and *LZC*, and classification using ANFIS.

Figure 2. ANFIS architecture used in this study. The inputs to the adaptive network are the mean values obtained with *SampEn* (*x*) and with *LZC* (*y*).

Figure 3. ROC curves showing the discrimination between AD patients and control subjects with the mean values of *SampEn* and *LZC*.

FIGURE 1

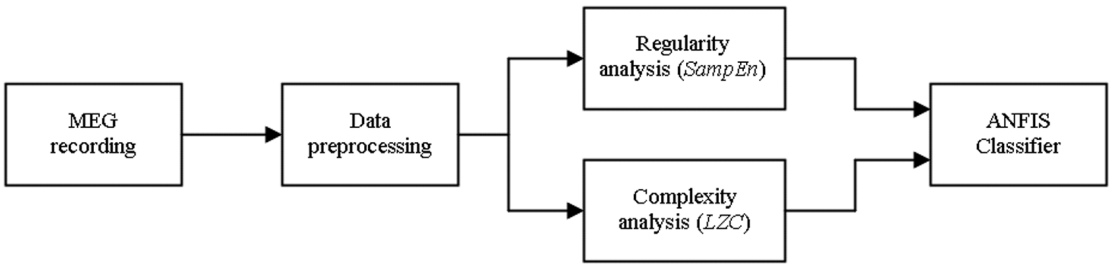
MEG recording
Data preprocessing
Regularity analysis (SampEn)
Complexity analysis (LZC)
ANFIS Classifier

FIGURE 2

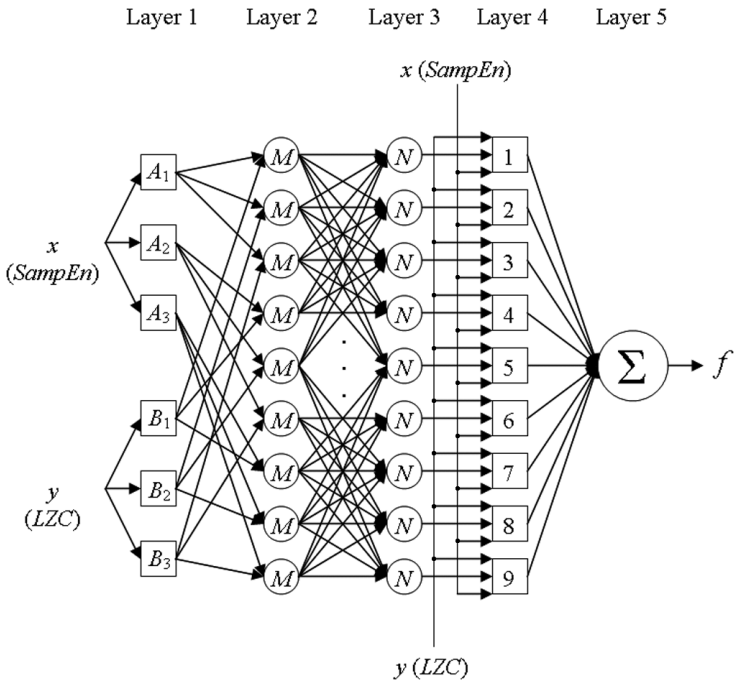
Layer 1
Layer 2
Layer 3
Layer 4
Layer 5
x (SampEn)
x (SampEn)
y (LZC)
y (LZC)
A1
A2
A3
B1
B2
B3
M
N
1
2
3
4
5
6
7
8
9
Σ
f

FIGURE 3