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# Network analysis of foramen ovale electrodes recordings in drug-resistant temporal lobe epilepsy patients.

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| **Abstract:** | Approximately 30% of epilepsy patients are refractory to antiepileptic drugs. In these cases, surgery is the only alternative to eliminate/control seizures appearance. However, a significant minority of patients remain with post-operative seizures, even in those cases where the suspected source of seizures has been correctly localized and resected. The protocol presented here combines a clinical procedure routinely employed in the pre-operative evaluation of TLE patients with novel techniques of network analysis. The method allows evaluating the temporal evolution of mesial network parameters. Inserting bilaterally foramen ovale electrodes (FOE) into the ambiens cistern allow recording simultaneously electrocortical activity at several mesial areas of the temporal lobe. A network approach applied to the recording time series allows to depict the temporal evolution of the mesial networks, both interictal and during the seizures. In this way, the presented protocol offer a unique way of visualize and quantify measures which take into account the relationships between several mesial areas, instead of a single one. |
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**TITLE:**
Network analysis of foramen ovale electrode recordings in drug-resistant temporal lobe epilepsy patients

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**SHORT ABSTRACT:**
This protocol describes a procedure to track the evolution of mesial network measures in temporal lobe epilepsy (TLE) patients. It is based on the combination of intracranial recordings with a novel numerical technique for data analysis. Specifically, we present a protocol for network analyses of foramen ovale recordings.

**LONG ABSTRACT:**
Approximately 30% of epilepsy patients are refractory to antiepileptic drugs. In these cases, surgery is the only alternative to eliminate/control seizures. However, a significant minority of patients continues to exhibit post-operative seizures, even in those cases in which the suspected source of seizures has been correctly localized and resected. The protocol presented here combines a clinical procedure routinely employed during the pre-operative evaluation of temporal lobe epilepsy (TLE) patients with a novel technique for network analysis. The method allows for the evaluation of the temporal evolution of mesial network parameters. The bilateral insertion of foramen ovale electrodes (FOE) into the ambient cistern simultaneously records electrocortical activity at several mesial areas in the temporal lobe. Furthermore, network methodology applied to the recording time series tracks the temporal evolution of the mesial networks both interictally and during the seizures. In this way, the presented protocol offers a unique way to visualize and quantify measures that considers the relationships between several mesial areas instead of a single area.

**INTRODUCTION:**
Epilepsy is a disabling disease that affects 1-2% of the world's population. In the majority of cases, seizures – the hallmarks of epilepsy – can be completely controlled or abolished with anti-epileptic drugs. However, approximately 30% of epilepsy patents are refractory to drug therapies. In the most common type of epilepsy, the temporal lobe epilepsy (TLE)[1], fortunately surgery is a valid alternative to improve the patient's condition. Results from meta-analyses show that almost two thirds of drug-resistant TLE patients are seizure-free in the first two to three years after the resective surgery[2,3], although this proportion varies across several factors, most notably, the type of hippocampal sclerosis[2]. A critical step for a successful outcome is the accurate localization of the so-called epileptic focus, the cortical area responsible for the generation of seizures, which is typically located in the mesial area of the temporal lobe. However, even in those cases where the epileptic focus has been correctly identified and resected during the surgery, a significant minority of patients either remains with post-operative seizures or must be placed under strict antiepileptic drug treatment to control seizures. Therefore, a new perspective has emerged. Attention is no longer focused solely on isolated areas, and cortical interactions now constitute the fundamental issue. This "network" approach is grounded in the connectome concept[4], which focuses attention in the neural connections between different areas rather than highlighting the role of compartmentalized structures. This new paradigm was found in graph theory, a mathematical framework devoted

to the study of topological and statistical properties of graphs, the appropriate tool to express its fundamental findings. Under this perspective, the brain is considered as a set of nodes interconnected by links[5-9] such that nodes are represented by the cortical areas covered by the electrodes and the links between them are given by the degree of synchronization. Thus, this network approach has been used in the analysis of invasive electrode recordings and has provided new information to promote the understanding of the underlying mechanism of seizure generation and propagation.

Among the many invasive neurophysiological techniques routinely employed in most epilepsy centers around the world, the foramen ovale electrode (FOE) is particularly remarkable. FOE is a semi-invasive technique because there is no need to perform a craniotomy, which reduces surgery-related complications[10]. Additionally, the location of FOE in the ambient cistern[11] makes them especially convenient for recording mesial activity from several cortical structures involved in seizure generation and propagation, such as the entorhinal cortex. Therefore, its use since its appearance is widespread in the presurgical evaluation of drug-resistant TLE patients. Traditionally, this technique is used to locate irritative activity in the form of interictal epileptogenic spikes and sharp-waves, and more importantly, to accurately identify the area of mesial seizure onset.

The new definition proposed from the Commission on Classification and Terminology from the International League against Epilepsy (ILAE) suggests that seizures originate at some point within particular networks[12]. Moreover, several studies have demonstrated that seizures are caused by abnormal network activity rather than by an isolated pathological area[13-16]. Clearly, this new perspective requires reanalysis of previously acquired information using new numerical methods, such as complex network methodology. Although the practical use of these analyses is still incipient in clinical practice, several research studies have demonstrated their value[13-17].

The protocol described below is the combination of a clinical practice routinely performed on drug-resistant TLE epilepsy patients with a novel technique of network analysis. The method allows for the evaluation of the temporal evolution of mesial network parameters. The bilateral insertion of FOE into the ambient cistern simultaneously records the electrocortical activity at several mesial areas of the temporal lobes. A network approach applied to the recording time series tracks the temporal evolution of the mesial networks both interictally and during the seizures. In this way, the presented protocol offers a unique way to visualize and quantify measures that considers the relationships between several mesial areas.

**PROTOCOL:**

In the protocol described below, steps 1, 2 and 3 belong to both the research and clinical protocols, which are both strictly followed from every mesial TLE candidate for resection surgery selected only by clinical criteria. Steps 4 and 5 belong exclusively to the research protocol. Both procedures are in accordance with the guidelines of the Ethical Committee of the Hospital de la Princesa.

**1. Pre-implantation Procedures**

1.1. Explain the experimental procedures to the participant, specifying which points correspond to the research and which ones are applicable to clinical practice, remarking that the research procedure is in no way modified from the clinical procedure. Pay special attention to explaining the potential risks of the surgical implantation of electrodes. Obtain a signed informed consent form the participant.

1.2. For all candidates for resection surgery, perform presurgical neurological and neuropsychological examinations[18].

1.2.1. Evaluate the patient by interictal single photon emission computer tomography (SPECT) with $^{99}$Tc-HmPAO, magnetic resonance imaging (MRI) 1.5 T and video-electroencephalography (v-EEG) using 25 scalp electrodes according to the 10-20 international system and Maudsley's protocol[18].

1.2.2. During the presurgical v-EEG recording stay, progressively taper the antiepileptic drugs from the second day to the fourth day (approximately one-third of the dose per day).

**2. Implantation Procedures (Surgery)**

2.1. Administer antiepileptic drugs pre-operatively, and perform surgery under general anesthesia (3 mg/kg propofol bolus, followed by 0.2-0.3 mg/kg fentanyl and 0.5 mg/kg rocuronium).

2.2. Insert two six-contact FOEs with a 1-cm center-to-center distance bilaterally into the ambient cisterns using Kirschner's technique[19].

2.2.1. Place the patient on the operating table in the supine position, with the neck gently extended at 15 degrees. Prepare the patient's cheek with an iodine solution, starting at the incision site and circling outward, and drape the area immediately surrounding the incision site.

2.2.2. Puncture the skin with a 20-gauge spinal needle according to Hartel's landmarks[20]: an entry point approximately 3 cm lateral to the ipsilateral side of the oral commissure toward a point immediately inferior to the ipsilateral pupil in the anterior-posterior plane and a point approximately 2.5 cm anterior to the external auditory meatus in the lateral plane.

2.2.3. Advance the needle toward the region of the foramen ovale under fluoroscopic guidance. Use the lateral views provided by the fluoroscopy images to determine the position of the needle tip. When the needle passes the foramen ovale, remove the stylet, replace it with an electrode, and advance it into the ambient cistern (**Figure 1A**).

2.3. Assess correct implantation by fluoroscopic imaging in the operating room[21]; this is important to exclude penetration into the foramina of the skull base, such as the inferior orbital fissure (located anterior to the foramen ovale) and the jugular foramen (located posterior to it).

Such misplaced cannulation could potentially lead to serious neurovascular injury[22].

2.4. Once the electrodes are correctly positioned in the ambient cisterns, secure them to the skin with drapes. Wake up the patient, and lead him or her to the recovery room.

**3. Acquisition of FOE recordings**

3.1. Return the patient to the v-EEG room for a stay of approximately 5.2 ± 2.4 days (mean ± SD).

3.2. Place 19 electrodes according to the international 10-20 system.

3.2.1. Measure the distance between the nasion (bridge of the nose) and the inion (occipital protuberance) using a measuring tape, and mark with a marker the middle point (location of the Cz electrode). Measure and mark the point 10% of the distance above the nasion (location of the Fpz electrode).

3.2.1.1. Repeat the same procedure for the inion (location of the Oz electrode), marking the distances 20% from the Cz in both the nasion and inion directions (locations of the Fz and PZ electrodes, respectively).

3.2.2. Measure the distances between both preauricular points, and mark the distances 10% above the left and right preauricular points (T3 and T4 electrodes, respectively). Then, mark the distances 20% above both T3 and T4 in the Cz direction to obtain the locations of C3 and C4.

3.2.3. Create a circumference using the measuring tape to link the Fpz and Oz at 5% of the distances above both electrodes at FP1 (left) and FP2 (right) in the front and at O1 (left) and O2 (right) in the back.

3.2.4. In the same circumference, add 10% of the distance upward in the inion direction to obtain the position of F7, add 10% to reach T3 (it should be located above the line between the preauricular points), and add another 10% to obtain T5 (O1 electrode). Mark each electrode position and repeat the same procedure for the right (even) electrodes.

3.2.5. Measure and mark the intersection (F3 electrode location) halfway between F7 and Fz and 20% of the distance upward from Fp1 in the F3 direction. Repeat this process in each quadrant of the head to obtain F4 (front-right position), P3 (back-left position) and P4 (back-left position).

3.2.6. Clean and dry the skin. Place a moderate quantity of collodion with conductive gel in each electrode cup, and position the electrodes in the prepped areas. Dry the collodion with compressed air.

3.3. Connect all of the electrodes (scalp and FOEs) by wires to the electrode box, which is already connected to an electroencephalographer. Ensure that the electrode signals are good,

and verify that the scalp electrodes impedances are under 10 kΩ using the electroencephalographer.

3.4. Acquire digital scalp electroencephalogram (EEG) data and FOE data at 1024 Hz using a video synchronized electroencephalographer (v-EEG), and filter the data using a band-pass filter in the range 0.5-100 Hz and a Notch filter (50 Hz) with the electroencephalographer.

3.5. Progressively remove the antiepileptic drugs from the second to the fourth day (approximately one-third of the dose per day) to increase the likelihood of seizures. This step depends on the particular drug prescription of each patient.

3.6. Use both interictal paroxysmal and ictal activities to approximately locate the ictogenic areas by identifying the electrodes/channel where epileptogenic elements appear[23], including the slow-wave complex, polyspikes, runs of rapid spikes, sharp waves, sharp-and-slow-wave complex, slow sharp waves, spikes and spike and slow waves. Record the times of seizure onset and end, as well as any other clinical signs or occurrences relevant to the study. There is a one-to-one mapping between the electrodes location in the patient's head and the head model in the EEG software which allows identifying anatomically where the epileptogenic activity appears.

3.7. When the study is finished, remove the FOEs at the v-EEG unit by gently pulling them out while the patient's mouth remains half-opened. Do not systematically perform imaging after FOE removal, except when neurological symptoms appear. In such cases, perform an urgent computed tomography (CT) scan.

**4. FOE signal preprocessing**

4.1. Export the data stored on the electroencephalographer at 200 Hz in ASCII format in epochs suitable for numerical analysis of approximately 30 min of seizure activity (already identified by an expert neurophysiologist) (**Figure 1C**). Avoid epochs containing artifacts, such as saturated electrical activity, muscle activity, and electrode displacements.

4.2. Open the exported files using any UNIX stream editor, and remove all non-numerical characters from the exported data files, leaving only time stamps and channel voltages. Save the modified files for further numerical analysis.

Note: From now on, perform all calculations using *R* packages from the *R* repository or homemade codes (**Table 1**).

4.3. Using *R* software, install the required *R* packages, and load the modified data files into the R environment. Order all channels, assigning each one to a particular column of the array that contains all of the data, eliminating empty channels and referencing them to an average mid-line reference (Fz + Cz + Pz)/3.

4.3.1. Use the Fast Fourier Transform algorithm (*R* function: fft) and plot the resulting variable

to check for the effective removal of the line frequency (approximately 50 Hz). Use the frequency domain to filter other spurious frequencies that may contaminate the signals.

4.4. Convert the loaded data to a multivariate time-series object (*mts*) of 28 columns -16 scalp and 12 FOEs - using the *R* function ts. Divide the *mts* object into non-overlapping temporal windows of 5 seconds each (1000 data points at 200 Hz) to reduce the file size and optimize computation time.

**5. Post-processing calculations (complex network analysis)**

Note: Calculate the measures described below in each temporal window, starting at 5 minutes before seizure onset (60 windows) and ending at 5 minutes after seizure onset (60 windows), with the aim of visualizing the temporal evolution.

5.1. Calculate univariate measures, spectral power, excitability and spectral entropy for each individual column/channel without considering the correlations between different time series.

5.1.1. Calculate excitability (S) for each voltage activity time series using a homemade code according to the equation proposed by Schindler[24] (see supplemental file). S>2.5 is considered epileptogenic, an empirically determined threshold[17,25,26].

5.1.2. For each activity time series, calculate the power spectral density using a homemade code for the Delta (> 0.5 Hz and <4 Hz), Theta (4-7 Hz), Alpha (7-14 Hz), Beta (14-30 Hz) and Gamma (>30).

5.1.3. Calculate Shannon entropy with a homemade code using the power spectral density of each time series instead of the corresponding probability time series. Average the individual spectral entropy (SE) values obtained for each channel over a set of electrodes. Shannon entropy is explained in the supplemental file.

Note: A decrease in SE should be interpreted as a decrease in the number of frequencies of the spectrum because SE is the entropy of the spectrum.

**5.2. Network measures**

Note: This section assesses the interactions between different time series of electrodes.

5.2.1. Calculate the functional connectivity between each pair of voltage time series in each temporal window using the absolute value of the linear cross-correlation coefficient computed at zero lag (*R* function: ccf).

Note: To eliminate non-representative values of synchronization, establish a threshold based on previous studies[17,20,26]. Use a threshold of 0.5 in this particular case.

5.2.2. Install the *igraph R* package[27]. Create an igraph object from the adjacency matrix (R function: graph.adjacency). Use the correlation matrix obtained in the previous step, specifying that the graph is weighted and undirected.

5.2.3. In each temporal window calculate the average path length (APL) (*R* function average.path.length) for the entire network (scalp+FOE), and for each of the four sub-networks: left scalp, right scalp, left FOE and right FOE. In exactly the same way, calculate the density of links (DoL) (*R* function: graph.density), modularity (Mod) (*R* function: modularity) and the Average clustering coefficient (ACC) (*R* function: transitivity).

5.2.4. Repeat the previous steps 5.2.1 through 5.2.3 using phase synchronization (homemade *R* code) as an estimate of functional connectivity instead of the cross-correlation function.

5.3. To represent the size effects in the variable changes, calculate the standardized mean difference (SMD) (*R* function from package MBESS: smd), between the preictal and the ictal stages as well as between the preictal and postictal stages.

5.3.1. Taking the preictal as the baseline, select thirty seconds (6 values) five minutes before seizure onset mark, as the preictal value. A similar temporal window of 30 seconds can be chosen during the seizure in order to quantify the change, respect to the preictal stage, by using the SMD.

5.3.2. In a similar fashion, select a temporal window of 30 seconds five minutes after seizure ends in order to quantify the change during the positictal stage (respect to the preictal stage).

**REPRESENTATIVE RESULTS:**

The final position of the FOE is in the ambient cistern, as seen in the axial and sagittal MRI (**Figure 1A upper panels**). The contacts of the FOE record electrical activity from several mesial structures of the temporal lobe (**Figure 1A lower panel**). After the surgery (**Figure 1B left panel**), the patient is dispatched to the video-EEG room, where scalp electrodes are placed according with the 10-20 system (**Figure 1B right)**. During the stay at the video-EEG room, the patient is continuously monitored, saving for further analysis scalp and FOE recordings, as well video and vital constants. A typical raw scalp and FOE signals (**Figure 1C**) show the appearance of a seizure at the left FOE and its spread to scalp and right FOE contacts.

Representation of the epileptogenic activity by using the excitability (S) (**Figure 2**) corresponding to the raw EEG recordings from **Figure 1C,** during the transition from the preictal to the ictal and postictal periods. Seizure onset is marked with a solid vertical line and time (x-axis) are referred to this point. A value of S (excitability) > 2.5 represented irritative or epileptogenic activity[17,25,26]. Higher excitability (reddish colors) appeared firstly with higher intensity on the left FOE contacts (LFOE). This result is concordant with a left mesial temporal lobe epilepsy as informed by an expert neurophysiologist.

Temporal dynamics of several network measures as well as the spectral entropy (**Figure 3**)

during the transition from the preictal to the ictal and postictal stages, corresponding to the same seizure displayed in **Figure 1C** and **2**. Seizure onset is marked with a solid vertical line and time (x-axis) are referred to this point. In this case, the network was built upon the whole set of electrodes, including both scalp and FOE. DoL and ACC values were higher during seizures, with a decrease in the APL and Mod, suggesting an increase in the overall connectivity. During this period also, lower levels of SE were observed and sustained after the excitability (dotted vertical lines) disappears.

The analysis of the network measures ACC, DoLs and APL and the SE for each FOE (right and left) **(Figure 4)** during the transition from the preictal to the ictal and postictal stages. Seizure onset is marked with a solid vertical line and time (x-axis) are referred to this point. The evolution of this measures correspond to the same seizure of **Figures 1, 2** and **3**. The ipsilateral (left) mesial ACC, DoLs and APL presented earlier and higher changes than the contralateral values, which could be explained by the location of the seizure onset zone in the left temporal lobe. In this case, Mod could not be calculated because no subdivisions were available.
A representative video of the functional connectivity (**Figure 5**) during the same seizure of **Figure 1, 2, 3,** and **4** presents a critical change just after the seizure onset (Time 0). At that point the connectivity between all the electrodes increase dramatically, as can be seen by an increase of the number of links and the thickness (intensity) of that edges. This increase starts between the left FOE at time 0.1 and 0.2, and spreads to the contralateral side before reaching the whole network.

**Table 1: R functions used for data processing**

**Figure 1: Foramen ovale electrodes.**
(A) Final position of FOE into the ambient cistern. Upper panels show an axial (left) and sagittal (right) MRI images displaying the FOE contacts location (white arrows). A human specimen (cadaver) with an inserted FOE (lower panel, contacts marked with white arrows). (B) FOE and scalp electrodes setup. Patients head just after the FOE insertion surgery (left panel) and during the video-EEG stay (right panel). (C) FOE and scalp recordings. Complex partial seizure from a left TLE patient (5 minutes after and before seizure onset). RFOE1-RFOE6 stands for right FIE#1 to #6 and LFOE1-LFOE6 stands for left FOE#1 to #6. Seizure onset is marked by a vertical red line and a white arrow head.

**Figure 2: Representation of a complex partial seizure from a left TLE patient quantified by excitability.**
The color scale quantifies the excitability level (S) for each electrode. The right foramen ovale electrode (RFOE) and left foramen ovale electrode (LFOE) represent the contacts of the right and left foramen ovale electrodes (y-axis), respectively. The x-axis marks the time (in minutes) relative to seizure onset (thick vertical line) as determined by an expert neurophysiologist.

**Figure 3: Entire network (scalp+FOE) measures from the same patient and same seizure from Figure 2.**

The average clustering coefficient (ACC), average path length (APL), density of links (DoLs), modularity (Mod) and spectral entropy (SE) for the entire network (scalp + FOE) are represented. The vertical dotted lines represent the excitability (S). The x-axis marks the time relative to seizure onset (thick vertical solid line). A moving average over ten consecutive windows is represented by a thick solid black line.

**Figure 4: Mesial measures of the same patient from Figure 2 and 3.**
The average clustering coefficient (ACC), average path length (APL), density of links (DoLs) and spectral entropy (SE) for both the left and the right foramen ovale electrodes (FOEs). The vertical dotted lines mark the excitability. The x-axis marks the time relative to seizure onset (thick vertical solid line). A moving average over ten consecutive windows is represented by a thick solid black line.

**Figure 5: Dynamic of connectivity pattern during a complex partial seizure.**
Links intensity is represented by the thickness of the edges. Times (lower numbers) are relative to the seizure onset (Time 0). Each frame is 5 seconds long. Left and right foramen ovale electrodes (L1-L6 and R1-R6) are represented by coral and blue circles, respectively. Left and right scalp electrodes are represented by orange and cyan circles, respectively.

**DISCUSSION:**
Traditionally, epilepsy was studied under a zone-oriented approach, which isolated the importance of particular areas, essentially the seizure onset zone, as the unique cause of seizures. Very recently, a true network approach that emphasizes the importance of interactions between cortical areas has been favored over the classical zone-oriented perspective[13-17,28]. However, the current body of evidence for epilepsy as a network disease is still highly fragmented, and more research is needed. The present work aims to reanalyze data provided by traditional methods as the FOE, under the complex network approach. The protocol presented here describes a step by step methodological procedure to perform a complex network and spectral analysis of semi-invasive recordings in TLE patients.
The application of the technique described above has demonstrated the usefulness of the network approach as compared with the more traditional localized or zone-oriented perspectives. In recent works[17,29] it was shown that, using the very same procedure as the one described here, an imbalance in the mesial connectivity in refractory TLE patients is apparent. Mesial connectivity is reduced in the ipsilateral side both during the interictal[29] and ictal[17,29] stages. This result could not be anticipated by looking solely at the areas where the epileptogenic activity arises. This somehow surprising result was also described by using network theories on fMRI signals[30,31]. Moreover, the application of the combined technique of FOE+network theory has shown the equivalence of mesial activity during seizures and under the effects of a promoter of epileptogenic activity, as it is the pharmacological administration of etomidate[32].

The technique described here is capable of detecting mesial network imbalance in short interictal recordings lasting at most one or two hours[29]. In this way, a drastic reduction in the analysis time and patient hospital stay could be achieved. In addition, from a therapeutic

perspective, the existing imbalance in TLE patients could be “resolved” by using chronically implanted (by neurosurgeons) devices, as much as the way it is done in deep brain stimulation.

To obtain optimal results using the information provided in this protocol, some issues should be considered in advance. First, the implantation of the electrodes should be performed by an experienced neurosurgeon because their incorrect placement could produce severe neurological consequences and misleading recordings. Furthermore, the selection of appropriate epochs for further analysis relies entirely on the neurophysiologist’s interpretation of the raw EEG; therefore, experience in clinical EEG analysis is mandatory. The data format of the exported files from the electroencephalograph depends on the particular brand; consequently, good programming skills are needed to adapt the scripts to different data formats. Finally, to ensure the reliability of the data, quality controls should be applied to the results. Overestimation and false positives are likely to appear when working with a high number of correlations. In such cases, statistical methods to improve sensitivity should be used. In this regard, it is important to establish a threshold in the correlations to discard values that are not representative of a true underlying synchronization. Thus, in this protocol, an edge between nodes *i* and *j* will be only considered to exist if the absolute value of the correlation between these nodes is greater than 0.5, a criterion previously employed[17,26]. Other thresholds in the range of 0.2 to 0.8 should be employed to verify similar results and to ensure a smooth transition from one threshold to the following threshold. In addition to thresholds, other methodologies may be used to obtain reliable results, such as Bonferroni correction or surrogate data testing. Moreover, when working with EEG data, it is important to keep in mind that brain networks are complex systems with non-linear dynamics; therefore, in addition to the linear correlation, other non-linear synchronization measures should be used to ensure the quality of the results, such as mutual information or phase synchronization[33].

Calculating connectivity directly from scalp electrodes, as it is partially done in this work, entails some risks. The main problem rest in the contamination effect due to volume conduction, always present with scalp recording. One way to overcome this issue is by working on the sources space, an appealing alternative employed by many researches. Another approach demands the use of measures of synchronization which minimizes the amplitude effects of contamination. By using the phase synchronization (also known as Phase Locking Value) we minimize the effect of volume conduction, as it was demonstrated in several works[34].

As in other invasive neurophysiological techniques, recordings from FOE cannot be obtained from control subjects, a fact that severely limits the use of certain research protocols. Data from FOE recordings provide valuable information about mesial temporal lobe activity[17,29,35], especially during lateralization to the epileptogenic side in TLE patients[33]. Compared with invasive techniques, the FOE technique is non-traumatic for the brain and involves relatively simple manipulation, and its recordings are of high quality over long periods of time[11]. Compared to MRI, FOE recordings provide better time resolution of electrocortical activity. In addition, many possibilities exist to explore measures other than those used in this work. These facts also increase the possibility of analyzing several biomedical recordings simultaneously. These advantages of FOE recordings combined with complex network and spectral analysis

make this technique a powerful tool for epilepsy research with potential applications in clinical practice.

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**DISCLOSURES:**
The authors have no conflicts of interest to disclose.

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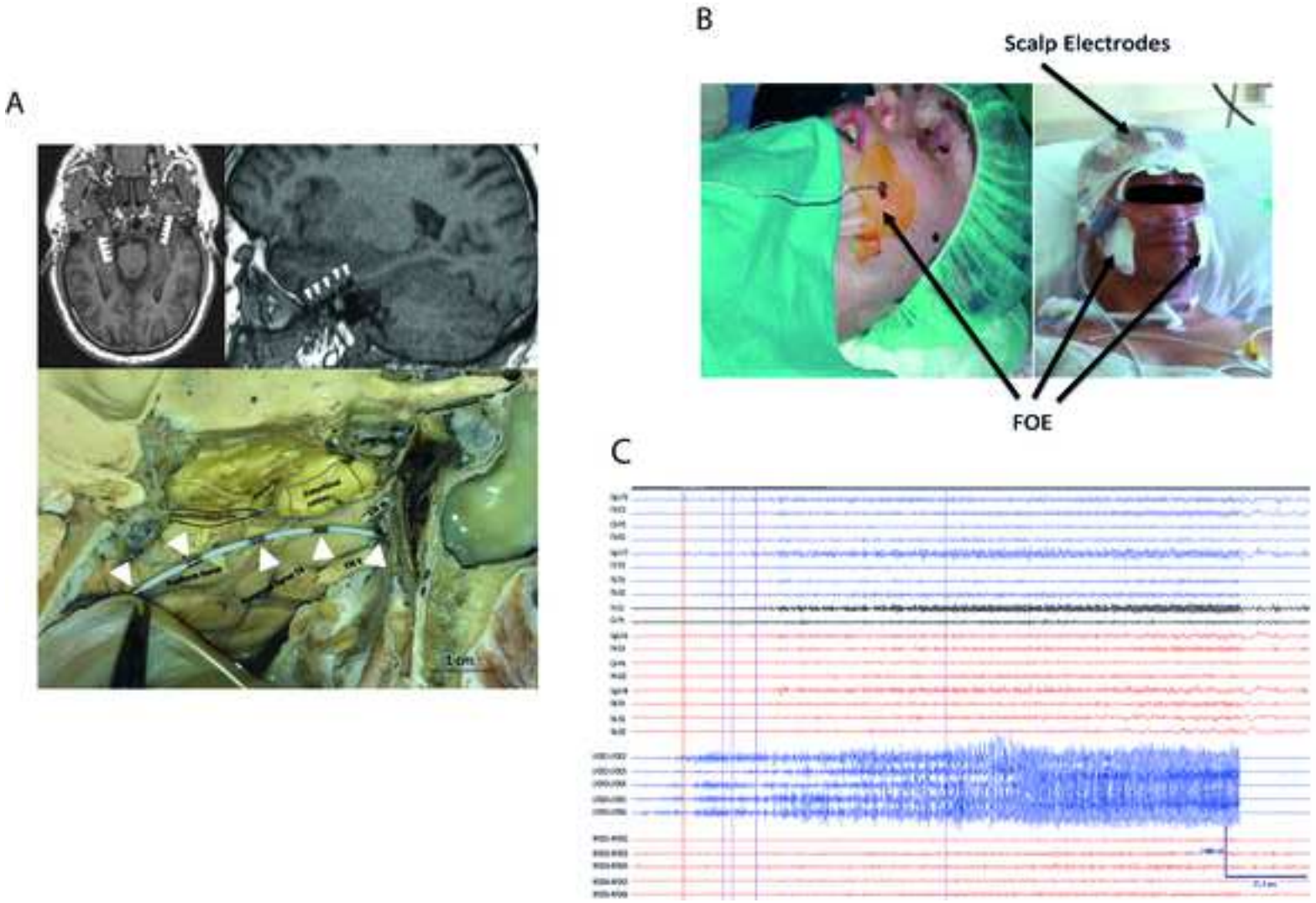

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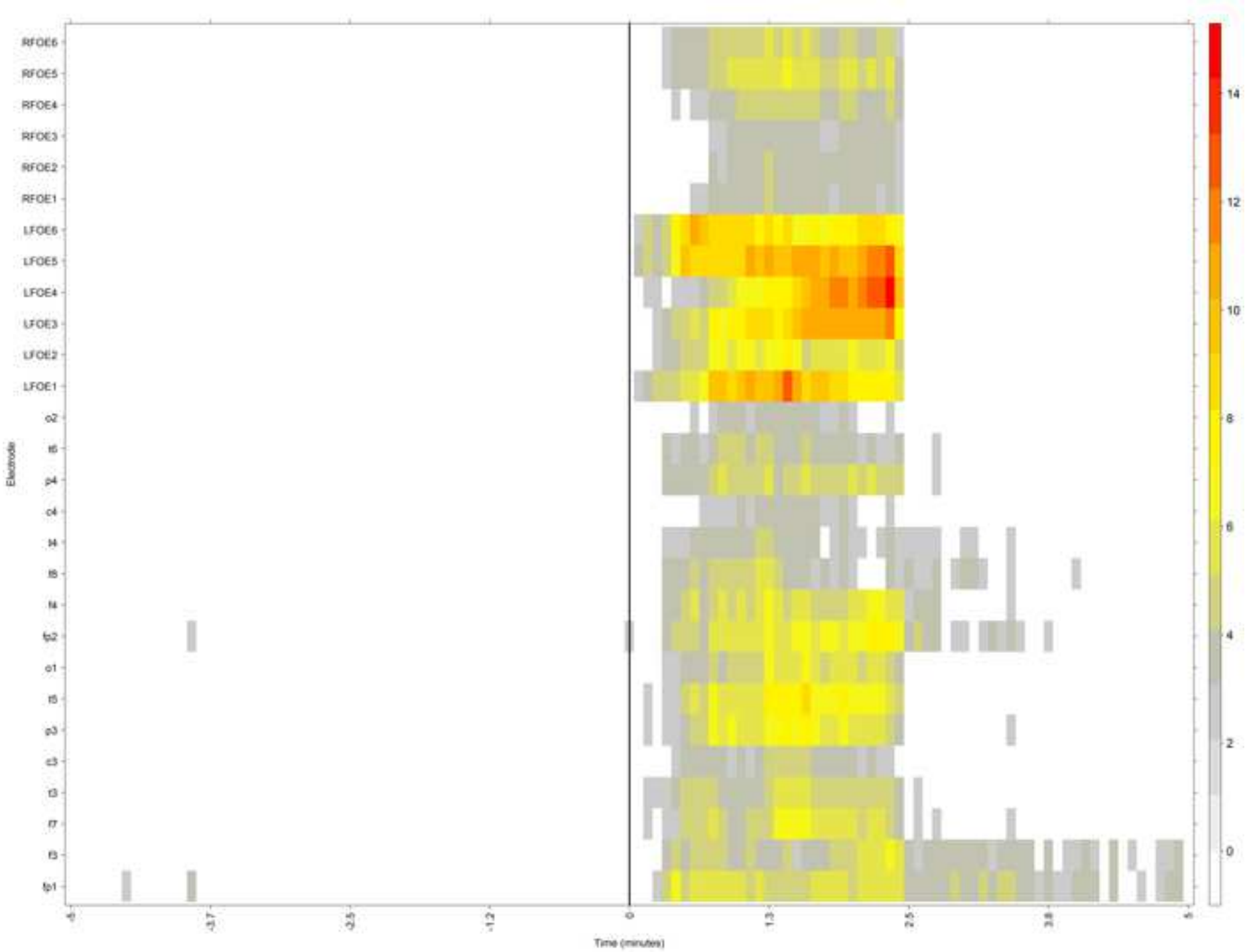

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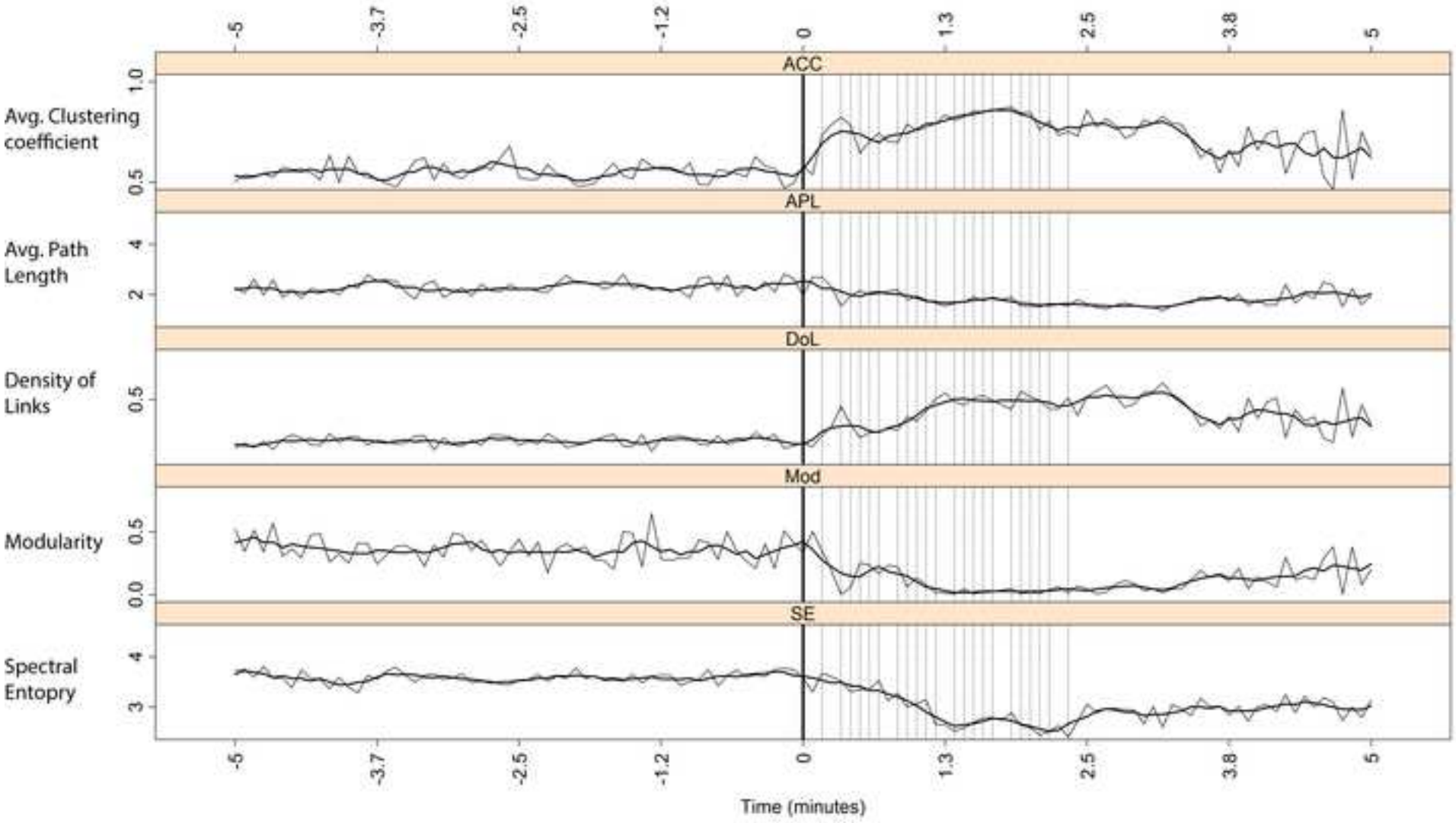

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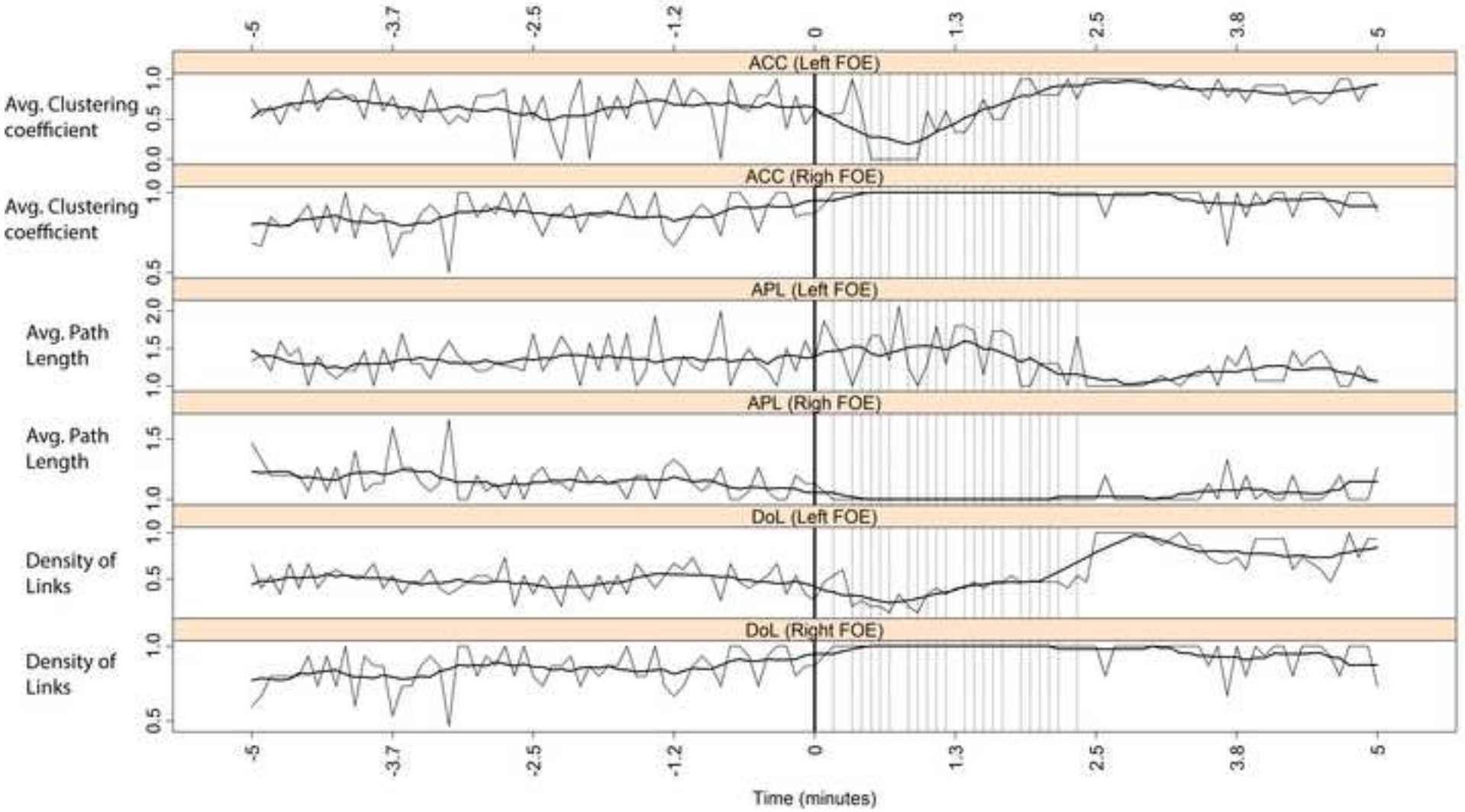

Table 1

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| Function | Protocol Point | Comments/Description |
|---|---|---|
| *fft* | 4.3 | (stats package) Computes the Fast Fourier Transform of a signal. |
| *ts* | 4.4 | (stats package) Creates a multivariate time-series object (mts). The sampling frequency should be provided. |
| Excitability | 5.1.1 | (homemade) function based on *diff* R function. Computes the absolute value of the slope of the signal and then normalize it to the standard deviation short baseline period. Threshold should be provided. |
| Power spectral Density and Spectral Entropy | 5.1.2 | (Homemade) function based on *spectrum* and *entropy* R functions. Compute the normalized power spectrum and the Shannon entropy of the normalized power spectrum |
| *ccf* | 5.2.1 | (base package) calculates the linear cross-correlation of mts object by using Pearson correlation at zero lag, generating a correlation matrix. Absolute values should be calculated. |
| *graph.adjacency* | 5.2.2 | (igraph package) Creates an igraph graph, the basic object used by the following igraph functions |
| *average.path.length* | 5.2.3 | (igraph package) determines the average path length of the graph, by computing the average number of steps along the shortest paths through all of the network nodes. |
| *graph.density* | 5.2.3 | (igraph package) Computes the densiy of links of the graph by computing the ratio between the actual number of links and all possible links of the network. |
| *modularity* | 5.2.3 | (igraph package) Determines the modularity of the graph, by computing which groups of nodes are more connected between them than with other nodes of the network |
| *transitivity* | 5.2.3 | (igraph package) Determines the average clustering coefficient of the graph, by computing the proportion of neighbors nodes that are also neighbor one of each other |

| | | |
|---|---|---|
| Phase synchronization | 5.2.4 | (homemade) function based in *fft* R funtion that calculates the mean phase coherence to obtain values between zero and one |
| *smd* | 5.3 | (MBESS package) Determines the standard mean difference -size effects- by computing the difference in mean between groups relative to the pooled differece |

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| Name of Reagent/ Equipment | Company | Catalog Number | Comments/Description |
|---|---|---|---|
| Foramen Ovale Electrodes | AD-Tech, Racine, USA | FO06K-SP10X-000 | Six-contact platinum |
| Electroencephalograph | XLTEK, Canada | XLT-EEG32T | Natus XLTEK |
| MRI machine | General Electric | | |
| SPEC machine | General Electric | | |

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Author(s): ANCOR SANZ-GARCÍA, LORENA VEGA-ZELAYA, JESÚS PASTOR, CRISTINA V. TORRES, RAFAEL G. SOLA, GUILLERMO J. ORTEGA

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Dear Editor, below you will find our answers to the Editor and Reviewers comments/concerns. Comments from reviewer #1 were copied down from the pdf document. Suggested changes made by both reviewers and the Editor were done using the track control. Our answers (in bold face) follow each editor/reviewer comment. In some cases however, we found some conflicts in accepting the reviewers' requests and, at the same time, follow the JoVE instructions for authors. In those cases, a compromise solution was implemented.

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The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (54746_R2_042516.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.

1. Additional detail is required:
-2.3 – How is fluoroscopic imaging performed? Please provide a citation.
**A reference has been provided in the text**
**Zampella, J.E., Brown, A.J., Azmi, H. Percutaneous techniques for trigeminal Neuralgia. In: Handbook of Stereotactic and Functional Neurosurgery. Gandhi D.C., Schulder, M. ed., Chapter: 34, doi: 10.1201/9780203912416.ch34 (2003).**
-3.2.1 – How are the distances measured and marked?
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-3.2.4 – Are electrodes placed at these positions? Or are marks made? Please specify.
**The Information required in these points is now included in the text**

-3.6 – How are the areas located? How are they recorded? More detail is required for this step to be filmed?
**This information is now included the text. During the film the head model will be seen in the computer screen.**

-Will the scripts/codes used be provided as supplemental files?
**The scripts used in most of the calculations are home-made and hardly illustrative, at least without a step-by-step and extensive explanation. Therefore, we provide a table with the R functions employed and explained.**

-4.5, 5.1.2 – Please provide a citation or the code as a supplemental file.
**See above**

2. Results:
-Please re-write the results section so that the figures are cited within the text [ie (Figure X)] with discussion of the data rather than formatting it as "Figure X shows…". This reads too much like a list of figure legends.
-Figures 3 & 4– Please label the y-axis.
**This section has been rewritten according with the reviewer suggestion**

Answers to Reviewer #1:

Note to reviewers: In some cases we found some conflicts in accepting the reviewers' requests and, at the same time, follow the JoVE instructions for authors. In those cases, a compromise solution was implemented.

1- (line 75) Very vague remarks...only in focal epilepsies especially TLE,surgery becomes a viable option...make adequate changes

**The paragraph was modified according with the reviewer suggestion**

2- (line 75) Surgical outcome needs to be commented a range quoting all important outcome studies in TLE after surgery.A single value of 66% is not acceptable.

**The paragraph was modified according with the reviewer suggestion and a new reference (3) was included.**

3- (line 83) Network hypothesis with references needs to be highlighted since this manuscript is dependent on the same.

**A new paragraph with 5 new relevant references (5-9) has been included in this new version of the manuscript, following the reviewer suggestion.**

4- (line 94) Although a time tested procedure,is FOE still in use and if so,is it being routinely used in any center?

**FOE are routinely used in our center as part of the protocol in some drug resistant TLE patients. FOE are actively used in other medical centers, see for instance reference:**

**Sheth, S. A., Aronson, J. P., Shafi, M. M., Phillips, H., Velez-Ruiz, N., Walcott B.P., Kwon, C.S., Mian, M.K., Dykstra, A.R., Cole, A., Eskandar, E.N. (2014). Utility of foramen ovale electrodes in mesial temporal lobe epilepsy. *Epilepsia, 55*(5), 713-724.**

5- (line 130) Why certain areas are highlighted yellow?

**Yellow areas will be included in the script of the film. They represent key issues of the work. It is an editorial request.**

6- (line 238) Has this methodology utilized eralier?

**Yes, the methodology showed in this work has been used in several earlier works, on either FOE or subdural electrodes, for instance:**

**Vega-Zelaya et al. Assessing the equivalence between etomidate and seizure network dynamics in temporal lobe epilepsy. Clin Neurophysiol. (2016) 127(1):169-78.**

**Vega-Zelaya et al. Disrupted Ipsilateral Network Connectivity in Temporal Lobe Epilepsy. PLoS One. 2015 Oct 21;10(10):e0140859**

**Vega-Zelaya et al. Inhomogeneous cortical synchronization and partial epileptic seizures. Front Neurol. 2014 Sep 24;5:187.**

**Ortega et al. Impaired mesial synchronization in temporal lobe epilepsy. Clin Neurophysiol. 2011 Jun;122(6):1106-16.**

**Also, other groups (on neurophysiological recordings) utilized network approaches:**

**Courtens et al. Graph Measures of Node Strength for Characterizing Preictal Synchrony in Partial Epilepsy Brain Connect. 2016 Jul 22.**
**van Diessen et al. Electroencephalography based functional networks in newly diagnosed childhood epilepsies. Clin Neurophysiol. 2016 Jun;127(6):2325-32.**
**Burns et al. Network dynamics of the brain and influence of the epileptic seizure onset zone. Proc Natl Acad Sci U S A. 2014 Dec 9;111(49):E5321-30.**
**van Diessen et al. Brain Network Organization in Focal Epilepsy: A Systematic Review and Meta-Analysis. PLoS One. 2014 Dec 10;9(12):e114606.**
**Varotto et al. Epileptogenic networks of type II focal cortical dysplasia: a stereo-EEG study. Neuroimage. 2012 Jul 2;61(3):591-8.**
**Wilke et al. Graph analysis of epileptogenic networks in human partial epilepsy. Epilepsia. 2011 Jan;52(1):84-93.**
**Schindler et al. Evolving functional network properties and synchronizability during human epileptic seizures. Chaos. 2008 Sep;18(3):033119.**
**Ponten et al. Small-world networks and epilepsy: graph theoretical analysis of intracerebrally recorded mesial temporal lobe seizures. Clin Neurophysiol. 2007 Apr;118(4):918-27.**
**This methodology has been the subject of several reviews:**
**Bernhardt et al. Network analysis for a network disorder: The emerging role of graph theory in the study of epilepsy. Epilepsy Behav. 2015 Sep;50:162-70.**
**Gleichgerrcht et al. Connectomics and graph theory analyses: Novel insights into network abnormalities in epilepsy. Epilepsia. 2015 Nov;56(11):1660-8.**
**van Diessen et al. Functional and structural brain networks in epilepsy: what have we learned? Epilepsia. 2013 Nov;54(11):1855-65.**
**Rubinov and Sporns Complex network measures of brain connectivity: uses and interpretations. Neuroimage. 2010 Sep;52(3):1059-69.**
**Boccaletti, S., Latora, V., Moreno, Y., Chavez, M., Hwang, D-U. Complex networks: Structure and dynamics. Phys Rep. 2006 424, 175-308.**
**Sporns et al. Organization, development and function of complex brain networks. Trends Cogn Sci. 2004 Sep;8(9):418-25.**
**And also, it has been used on fMRI data, for instance in:**
**Pereira et al. Asymmetrical hippocampal connectivity in mesial temporal lobe epilepsy: evidence from resting state fMRI. BMC Neurosci. 2010 Jun 2;11:66.**
**Bettus et al. Decreased basal fMRI functional connectivity in epileptogenic networks and contralateral compensatory mechanisms. Hum Brain Mapp. 2009 May;30(5):1580-91.**

7- (line 242) If epochs containing artefacts are excluded,won't the seizure onset of most seizures need to be eliminated because of the chewing and movement artefacts so common in TLE?

**As expressed in the main text of the manuscript, no artifacts were present in the analyzed recordings. Those recordings with artifacts, including muscle artifacts, were automatically discarded.**

8- (line 321) No details on how many patients or seizures were included in analysis. What are the inclusion and exclusion criteria or do all the patients undergo the same if they are TLe?
**The results presented here are representative results from only one patient. The aim of the manuscript is a methodological one. In this sense, number of patients and/or seizures and therefore the inclusion and exclusion criteria do not apply. These variables, however, can be consulted in the manuscript's bibliography, over which the described methodology is grounded.**

9- (line 394) Such redundant statements can be avoided since they do not add anything to this study,,non-traditional points of view...means????
**According with the reviewer suggestion, this paragraph has been deleted.**

10- (line 409) Include the authors' new innovations which has come up from this study in detail rather than a rambling discussion on what is to be done and what not?
**The inclusion of methodological steps into the Discussion section is an explicit requirement stated in the Instruction for authors of JoVE. In any case, a whole new paragraph was introduced (Discussion) regarding the innovations presented in the manuscript, as suggested by the reviewer: "The application of the technique …"**

11- (line 417) What has this experiment added to the already existing armementarium of tools in presurgical evaluation of TLE other than a routine FOE needs to be highlighted
**A new paragraph was introduced in the Discussion highlighting the importance of network over traditional analysis over FOE recordings: "This result could not be anticipated …"**

12- (line 432) Explain how it substantiates or refutes the network theory?
**See above (points 10 and 12)**

13- (page 14) ?? Legend to figures
**Legends of figures were in lines 358-380 (former version).**

14- (page 14) Figure B do not add anything
**Figure B has been changed**

15- (page 14) Figures are very poor quality
**Figures quality has been improved (300x300dpi) in this new version of the manuscript.**

*Major Concerns:*
N/A
*Minor Concerns:*
N/A
*Additional Comments to Authors:*
N/A

Answers to Reviewer #2:

Note to reviewers: In some cases we found some conflicts in accepting the reviewers' requests and, at the same time, follow the JoVE instructions for authors. In those cases, a compromise solution was implemented.

*Manuscript Summary:*

In this study, authors combined a traditional clinical protocol with a mathematically-sounded network analysis methodology to study the origin and propagation of epileptiform-like activity in the brain of epileptic patients. The paper is well-organized and discusses an interesting growing research area in clinical neuroscience, i.e. "the concept of pathological brain networks". I have some major comments that need to be address in order to recommend this paper for publication in JoVE.

*Major Concerns:*

1- Quality of figures is bad. Figure 1A need to present clearly the location for FOE as well as well define anatomical landmarks. The head of the patient with all electrode sets should be magnified in Figure 2B. Please, select attractive segments with EIDs and ictal data for Figure 1C. Please, indicate each of them and also provide their equivalent EEG segments. Provide the original data for the segment in Figure 2.

**According with the reviewer suggestion we have improved the quality of figures, specifically: The upper part of Figure 1A has been replaced by two MRI displaying the FOE contacts (white arrow heads). In the lower part of Figure 1A the exact location of FOE contacts are displayed in a human anatomical specimen. Figure 2B shows now the head of the patient right after the insertion surgery (left panel) and during the video-EEG room stay. Figure 1C has been replaced by the original video-EEG recordings corresponding to Figure 2.**

**Besides that, the resolution of all the figures has been increased up to 300x300dpi.**

2- Is section 4.2 needed?

**Actually, it depends on the software employed to export data from the acquisition equipment and also the software employed to preprocess it. In any case, we explain the procedure as detailed as possible during this critical step.**

3- Purpose of 4.5 is unclear. Please, explain it

**Point 4.5 has been eliminated in this new version of the manuscript because does not add any significant information.**

4- Provide Schindler's equations in 5.1.1, as well as frequency range and Shannon's equation in 5.1.2

**Schindler's and Shannon's equations have been added to the supplemental file. Frequency ranges have been add to the text.**

5- Functional connectivity at the level of sensor has been currently criticized by the community. Most of researches recommend performing it at the level of the brain sources.

Please, explain why authors are using a sensor based analysis.
**Although reconstruction of brain sources (from electrodes recording activity) is tempting in some ways, it entails several difficulties. The first one, and perhaps the most important is the lack of a unique model of the reconstructed source space. Moreover, any suitable model is, to some extent, arbitrary. While we are certainly aware of the problems of inferring interactions from scalp electrodes locations, specifically due to volume conduction, we prefer to circumvent this issue by using measures of synchronization which avoids this kind of contamination, as for instance the phase synchronization (also known as Phase Locking Value). A new paragraph was introduced in this new version of the manuscript mentioning this important issue (Discussion section).**

6- Please provide a rationale for the selection of the window parameters in 5.3.
**The whole paragraph was rewritten. There is no underlying criterion in the selection of these parameters.**

7- The note in line 325 - Is this the only criterion for good recording condition? I found it very inaccurate.
**Of course the criteria employed to ensure a correct implantation and good recording conditions are much more involved that the one expressed in a simplified phrase used former version of the manuscript. In order to avoid misunderstandings, we have eliminated this phrase from the manuscript.**

8- The note-like conclusion in line 350 should be removed since the authors only show the data for a single patient.
**The conclusion from line 350 has been deleted.**

9- Lack of discussion about the results from the network analysis. This is the main objective of this paper, so it has to be discussed and compared with other groups doing the same analysis in epilepsy. Based on the above obtained network, I would like to see a discussion whether a decrease in the weight of the edges or a rupture of edges or a change in the topology of the network are driving the triggering of the seizure. It would be very helpful to translate the methodology into a guidance for surgeons. They computed most of the important network features by using the R software, however they do not provide values neither discuss among these features which one might be the best or worst predictor.
**Because the manuscript's aim is a methodological one, we focus discussion on this particular issue, describing as detailed as possible all of the potential caveats one may encounter. In order to show the potential usefulness of the presented approach, we included a new paragraph summarizing some of the main results previously obtained by using this methodology. The cited bibliography in this new paragraph can be accessed for particular issues encompassed in the methodology.**
**Regarding the neurosurgeon issue requested by the reviewer, we have to mention that no surgery therapy is hypothetically envisioned by using this kind of analysis. However, a potential therapy using electrical stimulation could be implemented in order to balance the detected synchronization impairment. We have added a new sentence in this direction, according with the reviewer suggestion ("from a therapeutic point of view …")**

10- I would like to see a schematic representation of the anatomical network obtained from this procedure for this particular patient, with an explicit mention with edges are carrying the largest weight in the obtained weighted network. A discussion about postsurgical results will be also required.
**A video Figure (Figure 5) has been added in order to show the dynamics of the anatomical functional connectivity during seizure. See the preceding point regarding post-surgical results.**

11- They mention a threshold value for the correlation coefficient (Pearson correlation between two time series) to be used as a mark to consider two areas connected; however I could not identify any value neither the justification of it. They just provided the procedure but missed the inclusion of values. At what level of statistical significance they are working is missed also.
**The threshold value applied in the particular case of the manuscript is stated now (Note of point 5.2.1). A threshold of 0.5 with (typically) 1000 points in each time series yields for the correlation a p-value less than < 0.00001. More involved details of thresholds and statistical significance can be consulted in the references, in particular: Vega-Zelaya, L., Pastor, J., de Sola, R.G., Ortega, G.J. Disrupted Ipsilateral Network Connectivity in Temporal Lobe Epilepsy. *PLoS ONE* 10 (10), e0140859, doi: 10.1371/journal.pone.0140859 (2015).**

12- Regarding the point 3, it is common to use the Akaike Information Criterion along with the cross correlation coefficient in order to guarantee the validity of the "connected areas". I could not find any discussion about that.
**No AIC was used because no linear regression models were used to calculate the Pearson coefficient between two different time series.**

*Minor Concerns:*
1- Line 134 - for -> from
2- Line 272 - five -> 5
**Both concerns have been solved.**

3- Regarding the bibliography, I would like to see references as the following:
- Fundamentals of Brain Network Analysis, A. Fornito, A. Zalesky, and E. Bullmore, AP Press (2016)
- Concepts and principles in the analysis of brain networks, G.S. Wig, B.L. Schlaggar, and S.E. Petersen, Ann. N.Y. Acad. Sci. 1224 (2011) 126 - 146.
- Complex network measures of brain connectivity: Uses and interpretations, M. Rubinov, O Sporns, Neuroimage 52 (2010) 1059 - 1069.
**References have been added to the manuscript.**

4- Few references are from recent years, most of them are from before 2011. Only one from 2015 and couple from 2011 and one from 2012, the rest are from before 2010.
**Recent references have been added.**

*Additional Comments to Authors:*
N/A