TRACING THE BRAIN SPACE:
METHODS FOR THE STUDY OF EPILEPTOGENIC NETWORKS

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## INDEX

0·FOREWORD-------------------------------------------------------------1
  0·1·INSTITUTION------------------------------------------------------1

1·INTRODUCTION--------------------------------------------------------1
  1·1·EPILEPSY---------------------------------------------------------1
  1·2·NEURAL NETWORKS------------------------------------------------2
  1·3·SEMIOLGY---------------------------------------------------------2

2·DIAGNOSIS METHODS--------------------------------------------------5
  2·1·EEG-------------------------------------------------------------5
  2·2·BRAIN IMAGING----------------------------------------------------6

3·TREATMENT-----------------------------------------------------------8
  3·1·ANTICONVULSANT THERAPY-----------------------------------------8
  3·2·SURGERY AND ALTERNATIVE THERAPIES-------------------------------9

4·PROJECT-------------------------------------------------------------11
  4·1·GOAL-------------------------------------------------------------11

5·METHODS-------------------------------------------------------------11
  5·1·PATIENTS---------------------------------------------------------11
  5·2·EEG PROCESSING---------------------------------------------------12
  5·3·NODE LOCALISATION-----------------------------------------------13
    5·3·1·TOOLS--------------------------------------------------------14
    5·3·2·IMAGES AND MODELS-------------------------------------------14
    5·3·3·MEASURING SPACE IN THE BRAIN--------------------------------15
    5·3·4·EXPLORED BRAIN REGIONS-------------------------------------17
    5·3·5·STATISTICAL ANALYSIS----------------------------------------17

6·RESULTS-------------------------------------------------------------18
  6·1·EPILEPTOGENIC NETWORK DYNAMICS---------------------------------18
  6·2·EXTENT OF THE EPILEPTOGENIC NETWORK------------------------------19
  6·3·EXPLORED BRAIN REGIONS------------------------------------------20

7·CONCLUSIONS--------------------------------------------------------23

8·BIBLIOGRAPHY--------------------------------------------------------24
0. FOREWORD

The following project is product of my experience in the Epilepsy Unit of Hospital del Mar. It started as a period of my curricular practices but when it finished, the collaboration continued. I would like to thank the Epilepsy Unit, and Alessandro Príncipe in particular, with whom I was in direct contact during the development of the project.

0.1· INSTITUTION

The Epilepsy Monitoring Unit is a national reference centre awarded by the National Ministry of Health for refractory epilepsies and is member of e-epilepsy (http://www.e-epilepsy.eu/), the pan-European network of epilepsy surgery centres. The unit disposes of four beds for long-term video-EEG monitoring and is equipped with several stereo-electroencephalography (sEEG) acquisition systems of 128/256 channels and samplings rates up to 16 kHz. More than 100 evaluations are performed a year in candidates for epilepsy surgery with diverse diagnosis. In 2013, the Epilepsy Monitoring Unit pioneered the use of the first robotic system for electrode implantation in Spain (ROSA Robot) [1]. More than 12 implantations with sEEG are performed a year, being the centre with most accumulated experience in Spain. The number of epilepsy surgeries has increased in recent years reaching currently 40 cases per year.

1. INTRODUCTION

Epilepsy is one of the most prevalent neurological disease [2], imposing an important toll in terms of mortality and morbidity [3], especially among young subjects [4-5] A third of epileptic patients do not fully respond to anticonvulsants and roughly half of them can be further treated with surgery [6].

1.1· EPILEPSY

When a brain network undergoes an uncontrolled dynamic behaviour it may produce epilepsy. This altered processing also produces neurocognitive problems that depend on the affected network. So far, the idea has been that seizures, which are episodic interruptions of the normal cerebral activity in favour of highly synchronous discharges of neuronal populations, produce and worsen the cognitive deficit by progressively depleting or altering the recruited cells. Even though this is certainly true, it has also been demonstrated that some of those impairments do not necessarily depend on seizure frequency but represent another outcome of that uncontrolled behaviour [7]. Using a metaphor and assuming that the brain might be compared to a computer,
we would describe it as an assembly of several processing units. If one of them started to malfunction it might not only produce data processing errors, but also fatal freezes of the overall system. Restoring that unit would not only recover the normal data flow but also prevent those interruptions.

1.2 NEURAL NETWORKS

The brain is composed of around $10^{11}$ neurons and each neuron has an average of $10^4$ connections. This huge and complex net modulates its activity by electrochemical signals, based on the liberation of molecules called neurotransmitters (NT). This liberation is regulated by the equilibrium on the internal and external cell concentration of potassium, calcium and sodium ions.

The most common excitatory and inhibitory NT in the central nervous system are Glutamate and GABA respectively. The logic gate between Glutamate neurons and GABA neurons is negative feedback. When the concentration of Glutamate in the synaptic space exceeds a threshold, GABA is released, inhibiting the Glutamate liberation. The parameters of the negative feedback between the neurons determine the network electrical state. The alteration of this negative feedback at times is the origin of the network synchronization and the initial of the seizure [8].

Many efforts have been done in order to determine the minimal sufficient area of the brain that has to be ablated in order to attain seizure freedom or a significant reduction of ictal events [9]. These efforts have progressively led the concept of ictal onset and epileptogenic zone to evolve towards the idea of epileptogenic network (EN). Despite the efforts to characterise the EN, few studies have gone beyond the quantification of the epileptogenicity of brain areas as independent units and expanded such quantification to the network level with a pragmatical approximation. Many approaches have tried to determine brain activity dynamics [10-12], connectivity both anatomical [13-14] and functional [15-16], and also to identify and even predict ictal events [17-19]. None of these strategies though could, at the same time, describe network dynamics throughout seizure preparation, highlight brain areas involved in such preparation, and provide a clear description of the interictal cycle.

1.3 SEMIOLOGY

Extensively studied, clinical manifestations are both the target and the hallmark of the pathology. As the cognitive loss is determined by the localization of the focus and the neural networks related to it, epilepsy has not an unique symptomatological pattern and obviously not an unique clinical intervention protocol. Therefore symptomatology is so important, since it gives the first diagnostic clue and, when contained, patients’ lives go back to normality. Therefore the International League Against Epilepsy (ILAE) proposed in 1981 a classification based on the symptoms of epileptic seizures [20].
Figure 2. 1981 ILAE epilepsy seizure classification

Partial (focal) seizures

Simple partial seizures (consciousness not impaired)
  With motor signs
  With somatosensory symptoms
  With autonomic symptoms
  With psychic symptoms

Complex partial seizures (impairment of consciousness)
  Simple partial onset followed by impairment of consciousness
  With impairment of consciousness at onset

Partial seizures evolving to secondary generalized seizures
  Simple partial seizures evolving to generalized seizures
  Complex partial seizures evolving to generalized seizures
  Simple partial seizures evolving to complex evolving to generalized seizures

Generalized seizures (consciousness impaired)

Non-convulsive
  Typical absences seizures
  Atypical absences seizures

Convulsive
  Myoclonic seizures (sudden and brief contractions around the hours of sleep)
  Clonic seizures (fast and repetitive muscle cycle of contraction and relaxation)
  Tonic seizures (sudden and permanent muscle contraction)
  Tonic-clonic seizures
  Atonic seizures (loss of muscle tone and slumping to the ground)

Unclassified epileptic seizures (Inadequate or incomplete data)

Status epilepticus (Prolonged or repetitive seizures, with no time of recovery)

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1 The classification has two other sections: EEG seizure type and EEG interictal expression, but for the project goal it is enough.
Grouping symptoms into similar patterns gives the option to establish in every case, a general line of actuation. Once the symptoms are catalogued they can be related to a cause and help design an optimal intervention. In complementation to the symptoms classification, the ILAE issued in 1989 a new classification based on the topography and etiology of the epilepsy [21].

### Figure 3. 1989 ILAE epilepsy classification²

#### Related with the localization (focal, local, partial)
- Idiopathic
- Symptomatic
- Cryptogenic

#### Generalized
- Idiopathic
- Symptomatic/Cryptogenic
- Symptomatic
  - Unspecified etiology
  - Specified syndrome

#### Undetermined localization
- Generalized and focal seizure
- Misleading data

#### Special syndrome
- Seizure related with a situation

The first criteria determine the extent of the seizure, as it is defined in the previous section. The second criteria determine the cause of the seizure, dividing them in three groups: idiopathic (undetermined cause), symptomatic (determined cause), cryptogenic (hypothetic determined cause, lack of evidence). One more classification parameter can be added in terms of the status of the neural network and complementary tests: lesional or nonlesional.

These classifications are internationally recognised and applied with good results in clinical studies all over the world [22-24]. However, with the exponential extension of the studies, and the improvement of the diagnostic technology, some ambiguities and defects appeared. Part of the medical community wished for a restructuration [25-26]. ILAE published in 2010 a revised classification of seizures (maintaining the dichotomy partial-generalized and restructuring the subsections) and epilepsies (removing the topographic criteria, changing the etiologic ones, and

²The classification has subsections into every etiology section not showed in this figure, but for the project goal it is enough.
adding a new grade: syndromic specificity) [27]. The clinical community received the new classification with some rejection, being in disagreement with some criteria and highlighting ambiguity and confusion [28]. Nowadays, the most used classification is the one of the 1989, albeit the new one is gaining momentum. In complementation with the molecular and network definition, they guide the clinicians in the study and treatment of epilepsy.

2· DIAGNOSTIC METHODS

Precise diagnostic methods are required not only for guiding the surgery, that is a complex approach and may cause important functional disabilities, but also to determine patient populations that can become subject of studies testing novel strategies and medical treatments. In this section we will enumerate the most used techniques of both functional and anatomical diagnostic approaches.

2.1·EEG

Electroencephalography (EEG) is a monitoring method to record the electrical activity of the brain. Based on the signal recorded on the scalp skin, it measures the voltage changes between the explored areas produced by polarization and depolarization of the underlying neural populations.

The 10-20 system is an internationally employed method used to determine the position of the electrodes on the scalp. Composed by 21 electrodes, the distance between electrodes is 10% of the total nasion-inion (front-back) distance of the patient in sagittal direction, and 20% of the total right-left distance in coronal direction. Odd numbers label the left hemisphere, even numbers the right. Depending on the region, letters define the electrodes: Fp (frontal polar), F (frontal), T (temporal), C (central), A (earlobes), P (parietal), O (occipital).

![Figure 4. Epileptic electrical activity in EEG monitoring and position of electrodes in the scalp](image)

When a seizure starts, the electrical activity of some electrodes will increase abnormally, and depending on the connectivity, the electrical activity of other electrodes can increase as well after a varying periods of time. Many times but not always, through this test it is possible to detect where the seizure starts and where it propagates. Analyzing the type of epileptic electrical activity, the epilepsy can be further classified complementing the symptomatology (section 1.3) [29].

To achieve higher precision, an invasive EEG (iEEG) recording can be used [30]. Subdural electrodes (introduced over the surface of the brain) or depth electrodes are implanted, depending if the epileptogenic focus is on the neocortex or in deeper parts of the brain.
Default subdural electrodes are structured as grids (square or rectangular matrixes) of circular shaped (common diameter: 3 cm) contacts separated by 1 cm between each other either vertically and/or horizontally.

Depth electrodes are flexible plastic lines composed by 5-18 electrical contacts, separated by 3.5 mm. They can record the electrical activity at different depths. Using either or both this kind of implants it is possible to record the electrical activity of brain regions with great detail and precision.

![Figure 5. Subdural an depth electrodes and his implantation [30-32]](image)

Apart from the visual inspection of pathological electrical activity patterns, in epilepsy research and diagnosis, several signal processing techniques have been developed, one of the most common being the study of event related potentials (ERP). This technique averages the EEG activity to check for common patterns as response to sensory, motor or cognitive events [33]. Other techniques involve other kinds of feature extraction and/or averaging.

2.2 BRAIN IMAGING

Neuroimaging is a field with various methods to image the anatomical and functional structures of the brain. During the diagnostic procedure, along with the EEG, it gives crucial information about the location of lesions and the overall brain anatomy. Two main methods are used in epilepsy surgery.

Certain atomic nuclei can absorb and emit radio frequency energy when placed in an external magnetic field. In clinical and research MRI, hydrogen atoms are most-often used to generate a detectable radio-frequency signal that is received by antennas in close proximity to the anatomy being examined. Hydrogen atoms exist naturally in people and other biological organisms in abundance, particularly in water and fat. For this reason, most MRI scans essentially map the location of water and fat in the body. Pulses of radio waves are used to excite the nuclear spin energy transition and magnetic field gradients localize the signal in space. By varying the parameters of the pulse sequence, different contrasts can be generated between tissues based on the relaxation properties of the hydrogen atoms therein. Since its early development in the 1970s and 1980s, MRI has proven to be a highly versatile imaging modality. While MRI is most prominently used in diagnostic medicine and biomedical research, it can also be used to form images of non-living objects like the electrodes used for invasive EEG.
Functional MRI (fMRI) instead of the anatomical precision of the classical acquisition protocols of MRI, focuses on the brain activity produced by a certain event. When a brain region increases its activity the consumed energy goes along and the blood flow increases. In practical terms, the technique tracks the changes of blood flow that shadow the drift of neural activity. This kind of MRI imaging can be related to ERP studies [33].

Diffusion tensor imaging (DTI) studies the anatomical connectivity of brain regions. The movement of molecular water is restricted between the white and grey matter. Obtaining some diffusion parameters, the average angular direction of water can be estimated in each voxel (i.e., volumetric, practically cubic pixels that build up the whole image volume). Adding the fractional anisotropy parameter (FA), the level of diffusion can be determined. The final result is a map of the level of connectivity between different regions of the brain. The integration between tractography and EEG of the different regions in space-time gives a more detailed information of brain activity³.

Computed tomography (CT) produces a volume of data that can be manipulated in order to demonstrate various bodily structures based on their ability to block the X-ray beam. Although, historically, the images generated were in the axial or transverse plane, perpendicular to the

³ Despite it is not part of current project, we describe this technique because it will be part of the proceedings of this line of study.
long axis of the body, modern scanners allow this volume of data to be reformatted in various planes or even as volumetric (3D) representations of structures.

In epilepsy surgery CT is a good tool to obtain images of implanted depth electrodes. As the density of metal is higher than that of neural structures a 3D image of the cranium (bone’s most common element is another metal, calcium) and the depth electrodes implanted is obtained. The volumetric images generated by piling up the scanned slices are one of the best tools to analyze the position of electrode contacts.

Figure 8. Depth electrodes CT [37]

3. TREATMENT

Bromide therapy 160 years ago was the start of the treatment race against epilepsy. Since then, multiple medications were tested, at times without clear guidelines. Side effects were present and an integral and rigorous treatment guideline was necessary. Moreover the spread of such interventions was poor. In 2001, around 90% of epileptics in developing countries were not receiving the appropriate treatment [38].

In a joint effort between ILAE and the clinical community, a non-official guideline of first actuation in front of a suspected epilepsy was created. ILAE published in 2005 an actuation purpose [39] that combines with the knowledge and experience of the clinician. Over this initial intervention a monitoring protocol was built.

3.1. ANTICONVULSANT THERAPY

Antiepileptic drugs (AED) represent the cornerstone of medical treatment. Nowadays, over 36 AEDs are used in common and specialistic clinical practice. They are administered depending on the specific epilepsy etiology and patient characteristics (genetic background, age or gender). Side effects can be present, as nausea, vomiting, rash, fever, fatigue, or alteration of liver and pancreas metabolism [40]. AEDs are classified by action mechanism.
Figure 9. Mechanism action & AED examples

<table>
<thead>
<tr>
<th>Mechanism action &amp; AED examples</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium channel openers</td>
<td>Retigabine</td>
</tr>
<tr>
<td>Sodium channel blockers</td>
<td>Carbamazepine, Phenytoin</td>
</tr>
<tr>
<td>GABA receptor agonists</td>
<td>Diazepam, Phenobarbital</td>
</tr>
<tr>
<td>GABA reuptake inhibitors</td>
<td>Tiagabine</td>
</tr>
<tr>
<td>GABA transaminase inhibitors</td>
<td>Vigabatrin</td>
</tr>
<tr>
<td>Potential GABA mechanism of action</td>
<td>Gabapentin</td>
</tr>
<tr>
<td>Glutamate blockers</td>
<td>Felbamate</td>
</tr>
</tbody>
</table>

3.2. SURGERY AND ALTERNATIVE THERAPIES

Around 70% of patients respond to AED treatment and seizures can be fully controlled. Alternative treatments, always used in combination with AEDs and when the surgical option is not feasible, are:

- Ketogenic diet: Low in carbohydrates and high in fats, the metabolism of fats as the principal way to obtain energy produces as a residue acidosis and ketogenic bodies.

- Vagus nerve stimulation (VNS): Implantation of a stimulating device that sends pulses modifying the electrical activity of the vagus nerve, whose afferents reach, through various nuclear relays, the temporal and frontal lobes. This modulation approach is useful for some kind of epilepsies.

- Responsive neurostimulator (RNS): Implanted into the cranium, this device records electrical epileptic patterns and responds with pulses that disrupt the abnormal activity.

When patients do not respond to AED treatment they fall into the category of drug resistance. In this population, whenever possible (when a lesion is found and its ablation is safe) surgery is the best option. The technique is based on the ablation of the neural network responsible for the seizure priming.

Short and long term results of surgery are better than the ones of medical treatment [41-42]. Around 60% of patients are seizure free after surgery. Graphically representative of this is the review of the related literature between 1991 and 2005. Figure 10 shows the percentage of seizure free patients after temporal lobe surgery.

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4 The presence of ketogenic bodies would help to reduce seizures. The results of the use of ketogenic diet has been demonstrated empirically, but not with the same effects in all the patients.
The most used standard of postoperative classification outcomes of epilepsy surgery was proposed in 1993 [43]. Known as the Engel Epilepsy Surgery Outcome Scale, it divides the outcome in four classes and 2-4 subclasses:

**Figure 11. Classification of the outcome of epilepsy surgery [43]**

<table>
<thead>
<tr>
<th>Class 1</th>
<th>Free of disabling seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete seizure free since surgery (A), non-disabling simple partial seizures only since surgery (B), some disabling seizures after surgery but completely seizure-free for at least two years (C), and convulsions only when medications are withdraws (D)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Class 2</th>
<th>Almost seizure-free</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initially seizure-free but has disabling seizures now (A), rare disabling seizures since surgery (B), more than rare seizures after surgery but now rare surgery for at least two years (C), nocturnal seizures only (D)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Class 3</th>
<th>Worthwhile improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Worthwhile seizures reduction (A) or prolonged seizure-free intervals amounting to half the follow-up period, but not less than two years (B).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Class 4</th>
<th>No worthwhile improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No significant seizure reduction (A), no appreciable change (B), or seizures getting worse(C)</td>
</tr>
</tbody>
</table>
The classification has been generally accepted as useful and several studies corroborate its reliability [44-45], although the ILAE highlighted some disadvantages and proposed another classification [46].

4·PROJECT

Over the last decades, a number of analytical approaches have highlighted interesting features of the epileptogenic network, ranging from electrical (local) to anatomical and functional connectivity (global) patterns, allowing prediction (models) or warning (applications) about upcoming seizure. Despite the many efforts, the most clinically relevant solutions analyze the dynamics of brain areas separately to derive an anatomical description of the epileptogenic network extent.

This project is part of the scientific article Masters and slaves: the division of the network that opens the gate to seizures, not yet completed at the moment. The Project was under development during my stage at the Epilepsy Monitoring Unit (EMU) of Hospital del Mar [47]. In that project the authors try to answer some important questions of ictogenesis that will help researchers relate the electrophysiological activity to general neural network behaviour and anatomical localisation.

4.1·GOAL

In the aforementioned project a novel method to track brain activity dynamics is described. This method allows the identification of nodes that progressively take control of the network until ictal events occur. For the analysis Dr. Principe developed an algorithm similar to the estimators used in common data compression algorithms but adapting faster to dynamic changes. With the algorithm Dr. Principe and colleagues processed a feature depending on the synaptic activity of invasive EEG streams. More than 80 hours of Stereo-EEG (SEEG) recordings of refractory epilepsy patients that presented seizures distributed in clusters were analysed. Through the anatomical position of the nodes it was possible to calculate the extent of the epileptogenic network and relate it to the surgery outcome of the series of patients who underwent a minimal resection.

5. METHODS

A new method to track brain activity dynamics considering specifically the interplay between regions was developed in order to comprehend many possible relations, not only the synchronization between signals. The additive weighted context (AWC) is similar to the estimators used in common data compression algorithms (LZ78, LZMA). Like most of the latter, the AWC calculates the probability to find recurrent patterns, unlike them its memory adapts faster to context changes. To analyse the interplay between brain regions alphabets using a feature depending on the synaptic activity of invasive EEG streams were constructed. In practical terms, the AWC analysed items that resulted from the queued information of brain region pairs. Trying to predict the dynamics of all possible pairs, at each time step we built error matrixes that gave us information about the influence between regions.

5.1·PATIENTS

Twenty-four to 31 hours of SEEG recordings from 3 patients with pharmacoresistant focal dyscognitive seizures were analyzed. Patients were identified in the EMU among epilepsy surgery candidates who underwent SEEG evaluation for non lesional epilepsy. A summary of their characteristics and clinical facts is given in Figure 12. We included only patients who
presented all spontaneous seizures in a single cluster (maximum 6 hours between events) and who underwent a minimal surgical resection with very good outcome (minimum Engel is 1b) after at least three years. The recordings for each patient were selected as follows: we considered the whole time of the daily recording (in EMU each study is restarted every day for technical reasons) before the cluster, the whole cluster and some extra time, whose extension depended on contingency variables (start of electrical stimulation, temporary suspension of the recording for technical or clinical reasons).

All recordings were performed using a standard clinical EEG system (XLTEK, subsidiary of Natus Medical) with a 250-500 Hz sampling rate. A unilateral implantation was performed accordingly, using 5 to 8 intracerebral electrodes (Dixi Médical, Besançon, France; diameter: 0.8 mm; 5 to 12 contacts, 2 mm long, 1.5 mm apart) that were stereotactically inserted using robotic guidance (ROSA, Medtech Surgical, Inc).

The decision to implant, the selection of the electrode targets and the implantation duration were entirely made on clinical grounds without reference to this research study.

<table>
<thead>
<tr>
<th>Figure 12. Patient clinical and analytical data</th>
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</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Handedness</td>
</tr>
<tr>
<td>Personal history</td>
</tr>
<tr>
<td>Age at onset of epilepsy</td>
</tr>
<tr>
<td>Duration of epilepsy in years</td>
</tr>
<tr>
<td>Seizure frequency (episodes per year)</td>
</tr>
<tr>
<td>Aura</td>
</tr>
<tr>
<td>Intentional PET</td>
</tr>
<tr>
<td>SISCOM</td>
</tr>
<tr>
<td>Surgical Outcome</td>
</tr>
<tr>
<td>Follow-up</td>
</tr>
<tr>
<td>Neuropathology</td>
</tr>
<tr>
<td>Motor seizures (average per event)</td>
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<tr>
<td>Motor seizures within resection (%)</td>
</tr>
<tr>
<td>Average PEN mm (SD)</td>
</tr>
<tr>
<td>Average EEA mm (SD)</td>
</tr>
</tbody>
</table>

5.2- EEG PROCESSING

Since the focus of this EOG work does not encompass the whole project but focuses on the imaging results we will only briefly describe the methods used to track the brain activity dynamics.

The first step of data processing is the binary trending: the notch filtered EEG data is downsampled at a rate of 1 Hz then transformed into a series of zeroes and ones, using one when the previous sample is either equal or smaller than the current.

To build an error matrix EEG stretches of 1 minute have been used. All possible pairs of channel streams are transformed into binary trends and fed to the AWC, which returns the continuous
prediction error of both trends. Consider channel A and B, which are processed twice since the possible pairs are AB and BA, the average error of A and B are summed up and stored in a matrix, whose lines represent the average error made by each channel in predicting all other channels, while its columns represent the error made by all channels in predicting each channel.

To build the influence maps we use the error matrix of each time step. Each column represent the normalised influence level of the network nodes (the EEG channels) in a time unit (in our case a minute). We consider a node to have high influence when the error made to predict the trends of other nodes is low, while the error made by other nodes is high. Therefore, to calculate the influence value of a node at a time step, the sum of all column values of the corresponding error matrix is divided by the sum of all line values of the same node.

The compute the weighted influence of master nodes, the candidates are selected by cross-correlating the node influences with a linear function (linear growth likelihood). The top 5% of the nodes with linear growth are followed through time by computing their averages over 10 and 60 minutes and by subtracting the overall network influence values averaged with the same sliding windows (10 and 60 minutes).

5.3 NODE LOCALISATION

To perform anatomical, functional and electrical activity comparison of different brains, a normalized space coordinates is needed. The most used technique of normalization is based on the Montreal Neurological Institute (MNI) coordinates. To determine them researchers used an atlas of approximately 700 MRI scans taken across a big sample of normal patients and using different algorithms. Some landmarks, defined as the AC-PC line (traced from the superior surface of the anterior commissure to the center of the posterior commissure) and the edges of the brain were used as reference points to compute an average of the MRI scans. The result was a 3D normalized atlas, whose origin (0,0,0) was set in the anterior commissure [48].

The registration (superposition) of MRI scans of the patients on the MNI atlas translates and transforms every subject's space into a common one.

Figure 13. MNI coordinates: origin (0,0,0)
5.3.1· TOOLS

To perform calculations and area measures, image coregistration and normalization we used the Matlab IDE [49] and the Statistical Parametric Mapping (SPM) toolbox, designed for the analysis of brain imaging [50]. The FreeSurfer software was used for the segmentation of MRI images [51], while the SPM toolbox and the 3D Slicer software for the visualization [52] of both 2D slices and 3D models.

5.3.2· IMAGES AND MODELS

During the diagnostic procedures, several images are recorded. Pre-implantation MRI (3D) and post-implantation CT scan are used to build the model.

We used FreeSurfer to perform a whole brain segmentation of the pre-implantation MRI based on an atlas of the neuroanatomical structures [53], where each voxel of brain volume is assigned to one of 40 labels.

Figure 14. FreeSurfer atlas representation [51]

The processed MRI can be loaded into the SPM toolbox to perform the normalization and the CT coregistration. For this last step we use the MRI as fixed image to move the CT scan, that is progressively jiggled to reach the best positional match.

Notice that in some particular cases the SPM rigid coregistration algorithm cannot work as desired. In these cases, the coregistration can be performed with 3D Slicer. The registration module offers other several modalities like affine coregistration and linear transforms, where a matrix transform can be manually designed to initialize the coregistration process.

Coregistered images can be visualized with 3D Slicer, where sagittal, axial and coronal planes sections are visualized. Its model maker module is used to build the 3D model using the marching cubes triangles computation. Original CT scan image shows electrodes and all cables present in the space analyzed for the scanner.
Figure 15. 3D model without perform improving. Electrodes stuff is present

A threshold can be used to polish some unwanted details. Laplacian smoothing as well can improve the result. Once the 3D model is ready, the crop volume toolbox is used to box the image around the brain volume.

To add information to the 2D and 3D model, fiducials and rulers are placed to match the exact positions of electrodes. As rulers and fiducials can be given custom dimensions, they can easily mark the implanted electrodes and through their ticks also mark their contacts.

Figure 16. Ruler and fiducial

To obtain a volumetric representation of the resection we used the 3D Slicer software. We first coregistered the post-intervention MRI (p3DV) to the 3DV as if they were rigid bodies, since the resection and the craniotomies were minimal. After coregistration we subtracted the p3DV from the 3DV, smoothed the result and created a 3D model with the 3D Slicer Grayscale Model Maker.

5.4.2: MEASURING SPACE IN THE BRAIN

All images are normalized with Matlab. To work with SPM we have to convert the .nrrd image files (use in 3d Slicer) to .nii images files. The SPM toolbox uses the MNI atlas to perform the normalization and provides several template options (atlases). Obviously we chose the T1 option for MRI volumes.

The SPM interface is used to navigate the coregistered images to find the contacts. Coordinates appears in real-time over the orthogonal projection.

Due to the graphical resolution of the SPM toolbox, contact distinction can be difficult and at times even impossible because of the electrode displacement occurred after the application of the normalisation transform.
To overcome this problem, we measured the coordinates of the most medial and lateral contacts. Introducing both coordinates to a matlab function we created, we calculated the vector between them and divided it by the number of contacts. The function output is a table listing the coordinates of all the contacts of the analysed electrode.

Through the methods described below we could anatomically locate the master nodes. Once brain activity information was obtained, a predicted epileptogenic network (pEN) area could be calculated. We then analyzed its relation to the resected area and the excess (beyond the resection) explored area (EEA).

To calculate the accuracy of the virtual epileptogenic network we calculated the number of nodes falling into the resection. We related them to 105 surrogates defined as random nodes respecting the combinatory statistics of the EEG processing results. For every seizure, apart from a dynamic divided in an arbitrary number of bins a set of nodes is given. We used this set to derive the combinations and randomly calculate the surrogate nodes.

The pEN is defined as the area formed by the masters node of every electrode outside the resection and their distance from the closest projected point to the resection surface. To calculate it, the distance between every master node and his closest point is needed. After importing all volumes into the 3D Slicer software, by means of the orthogonal projection interface, we identified the closest projected resection point of every master node and measured its coordinates.

We designed a Matlab function to calculate the distance between master nodes of every electrode and their projected resection point as the module of the vector between them.

The EEA is defined as the maximum distance explored beyond the resection. To calculate it, instead of master nodes we used the coordinates of the most lateral contact of every electrode.
5.3.4· EXPLORED BRAIN REGIONS

To assess the brain areas explored by the SEEG we retrieved the coordinates of medial and lateral contacts of each and every electrodes and compared them with the MNI coordinates of standard cortical and subcortical groups and structures.

5.3.5· STATISTICAL ANALYSIS

Mann Whitney U and t-tests were performed to compare the average weighted influence of the preparation period of the 1st seizure and the final interictal time (6 bins), and the post-ictal and pre-ictal periods of the other seizures in all patients. To check for Gaussian distribution we used the Shapiro--Wilk normality test.

5 Red area is resected area
results
Only when the influence level of some nodes overcame the rest or, in other words, when the network was divided between masters and slaves, ictal events occurred. Seizures reset the cycle, allowing the same or other nodes, at times neighboring, other times functionally related, to restart the loop. Moreover, through the anatomical position of the master nodes we could calculate the extent of the EN and relate it to the surgery outcome of our series of patients who underwent a minimal resection.

6.1·EPILEPTOGENIC NETWORK DYNAMICS

Since the analysed period lengths differed between patients and between events of the same patient, in order to compare the dynamics between events and subjects we decided to compare the average influences of the same amount of stretches instead of opting for an arbitrary amount of time. Our hypothesis was that the master nodes’ influence would change before events independently from an arbitrary time unit. In other words, some events might occur before or later than others (reflecting the different phases of the seizure cycle) but the dynamics would not substantially change. Since events were separated by a wide range of time gaps (from minutes to hours), for statistical comparisons we opted for a binary solution: after the previous (postictal) and before the next seizure (preictal, see figure 20, bottom). Nonetheless we show all data (top) using 6 bins for cluster initiation and termination, and 4 bins for the seizure cycle. We present mean influence levels (line) and their standard deviation (halo), and use colors to highlight the seizure risk (red, higher; blue, lower).

As expected both cluster preparation and termination showed similar growing trends, at least in the last three bins, but at two different influence levels. Interestingly, the two trends started at the same pondered influence level (around 0), which indicates an influence steadiness between the leading nodes and the rest of the network, but with initial opposite tendencies. Considering postictal and preictal periods, we found statically significant differences of mean weighted influence levels between the initiation preictal (n=3) and the seizure cycle postictal (n=20, p<0.05) periods, between the postictal and the preictal periods of the seizure cycle (p<0.0005), and between the initiation preictal and both termination postictal (n=3) and preictal (n=3, p<0.05) periods.

From these findings it is possible to infer that when the leading nodes influence is counterbalanced by the rest of the network the seizure likelihood is practically zero and it starts increasing when the balance is broken.
**Epileptogenic network (EN) dynamics**: cluster initiation, cycle and termination. Weighted influence levels compared between events and patients: to compare EN dynamics between patients and events instead of computing the average of influence levels in a time unit we divided the time period of each event into an equal number of stretches and calculated the average weighted influence for each stretch. We show all data (curve graph, top) using 6 bins for cluster initiation and termination, and 4 bins for the seizure cycle, we show mean (line) and standard deviation (halo). We use 2 bins (postictal and preictal) for statistical comparisons (bars, bottom). Colors reflect seizure occurrence likelihood (red, higher; blue, lower). Error bars represent the standard deviation of master nodes weighted influence. *, p<0.05; ***, p<0.0005.

### 6.2 EXTENT OF THE EPILEPTOGENIC NETWORK

To calculate the extent of the predicted EN (PEN) and its relation to the real EN we used the resection limits that, in case of patient 1 and 3, who are seizure free, certainly contain the whole EN or its most important nodes. Since patient 2 still present auras we supposed that the performed resection at least overlaps with the real EN without covering it completely. Briefly, to calculate the extension of the PEN beyond the resected area we considered the master nodes positions outside it and projected them to the closest resection border; while to calculate the excess explored area (EEA) we considered the anatomical position of the last node of each electrode (see Methods for further details). Importantly, the extension of the PEN beyond the resection was significantly smaller than the EEA overall (p<0.001) and in each patient (Figure 12).
In figure 21 we show the positions of the PEN nodes. The ones depicted in bright red lie within the resected area, while the crimson ones lie outside. Interestingly, in patients 1 and 3 more than 50% of the PEN nodes were found inside the resected area, while only 23% of the calculated nodes were within the borders of patient 2 resection (Figure 12).

To understand whether the method could pinpoint real EN hubs we considered the nodes falling into the resection and compared them with surrogate nodes that were randomly selected within the same distributions patterns of the predicted ones. Despite the differences between patients and surgeries, significantly more predicted than surrogate nodes (p<0.05) were comprehended by the resections. Importantly, all master nodes linked to the cluster initiation were found to be within the resection margins.

**Figure 21. Epileptogenic network extent**

**Epileptogenic network (EN) extent:** to calculate the extension of the EN we considered both nodes that fell in and out of the resections, which we considered to contain the most important epileptogenic brain areas. **Line A. EN extent beyond the resection:** we calculated the distance in mm of nodes falling outside the surgical borders and compared them with the farthest contacts of each electrode. **Predicted hubs:** we considered that nodes falling within the resection should be considered real EN hubs and compared the number of them with surrogate nodes randomly selected but respecting the same distribution of the predicted master nodes. Error bars represent the standard deviation. *, p<0.05; **, p<0.001. **Line B. Anatomical position of the master nodes:** we show the master nodes selected with the top 5% time linkage threshold (bright red spheres indicate the nodes within, crimson spheres the nodes outside the resection margins represented as a red glass volume encased in the brain glass model).

**6.3· EXPLORED BRAIN REGIONS**

To define the MNI coordinates of the explored brain regions we normalized the pre-implantation MRI and the post-implantation CT scan with the Matlab toolbox SPM12. The orthogonal projections of the normalised CT are then inspected to determine tip and last contact of each electrode.
### Figure 22. Medial and lateral contact coordinates of every electrode

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7. CONCLUSIONS

A very simple feature extraction and a twist on a timeless concept explored through a machine learning technique especially created for parallel signal analysis allowed us to successfully track the network dynamics of seizure clusters and, at the same time, to topologically define the main hubs orchestrating the initiation of all ictal events in patients explored with SEEG and who underwent curative surgery. The experience tells us that not all seizures of a cluster are the same, nonetheless so far there has been no way to objectify this hypothesis. Despite that, we chose subjects who presented a thick and similar seizure distribution in the hope to find common features that could be related to the seizure occurrence risk, and also to track their anatomical localization.

With our analysis we could track the influence of all explored network nodes through a variable amount of time that ranged from minutes to hours. But more importantly we could find out that the interplay between nodes varied from seizure to seizure and that this interaction suggested a network that could not be fit into a simple three-dimensional shape. Indeed we could objectify that not all cluster seizures are the same and that some of them are driven by nodes that might be far away from the EN core but still functionally related. Despite this somehow expected result, which complicates the picture of the EN definition, more than half of the hubs were found within the boundaries of the performed resection in the two seizure free patients, while less than a quarter were contained by the resection of the patient who still present auras. The subjects were selected between patients who underwent SEEG evaluation and after surgery achieved a very good outcome in a follow-up period of at least three years. Apart from the seizure distribution and outcome, we selected those subjects who underwent a very selective surgery, in the hope that the resection would faithfully represent the real EN. The sporadic auras of patient 2 did not induce us to believe to find important differences in terms of hub distribution and relation to the surgical lesion. Indeed no significant differences in terms of the predicted EN extension beyond the resection margins were found between patients. Without doubt though the reduced number of hubs contained in the resection of the patient with the worst outcome and an undefined pathology (see Figure 12) suggests that the methodology might also help to anatomically define the EN, although this has to be confirmed with more patients, kind of epilepsies and etiologies.
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