UNIVERSITAT DE BARCELONA

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Jacint Sala Padró

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Jacint Sala Padró

Unitat d'Epilèpsia, Hospital de Bellvitge

Cognition and Brain Plasticity Unit, Universitat de Barcelona

Doctoral program in biomedicine-neuroscience

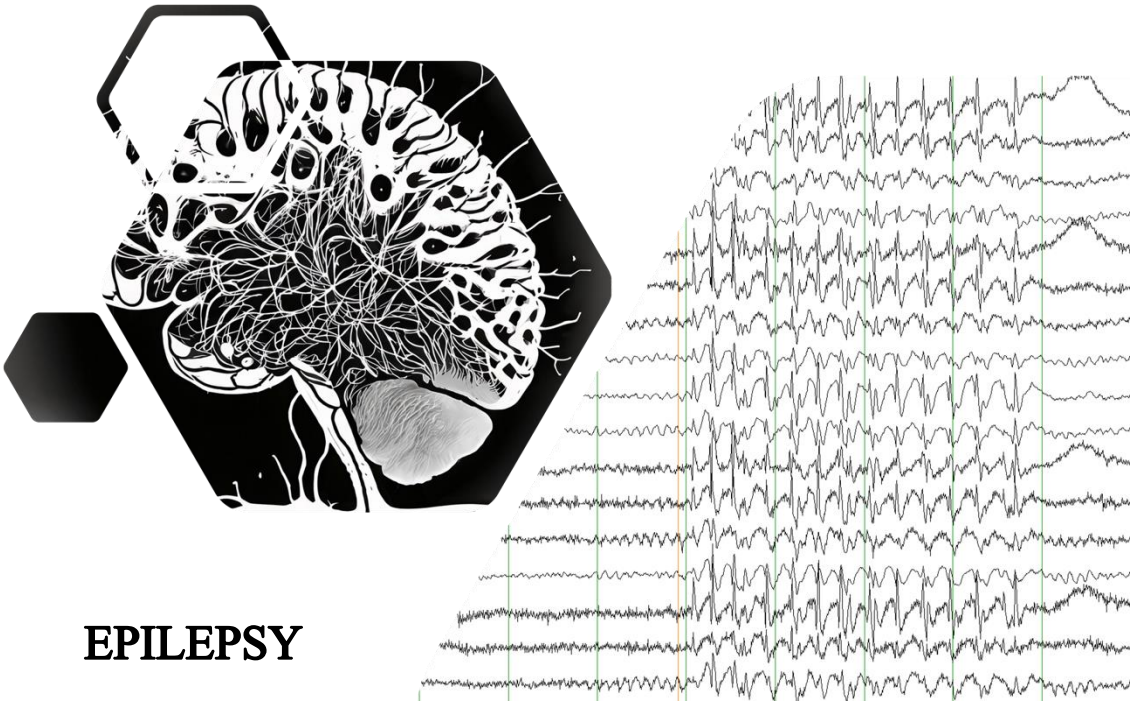
Supervisor:

Estela Cámara Mancha

Cognition & Brain Plasticity Unit

IDI BELL

Bellvitge Hospital Universitari



EPILEPSY

FROM A NETWORK PERSPECTIVE

AKNOWLEDGEMENTS

Aquesta tesis és el fruit de la feina feta al llarg dels últims 5 anys. Ara, escrivint les últimes línies, és impossible no pensar en tot i tothom el que d'alguna manera n'ha format part, amb idees, consells o senzillament aguantant-me en algun moment.

Suposo que per seguir el camí d'aquests anys, primer de tot donar-li les gràcies a l'Estela. Un dels primers records que tinc de quan ens vam conèixer és el bar de Bellvitge, i ja recordo estar comentant idees i projectes... Des del primer moment i fins ara! Espero que això segueixi així i tinguem molts més projectes pel futur, gràcies per la teva perseverança, les ganes de treballar i el perfeccionisme, no em paren de sorprendre.

Mencio apart també per la Mercè, per confiar en mi des de l'inici, per donar-me tot el que està al seu abast i per ajudar-me en el camí. També per ser-hi sempre, per les idees i els consells, i per la seva dedicació a la feina.

Sens dubte, hi ha hagut molta més gent que ha participat en la tesis i, potser el més important, he tingut la ocasió de conèixer i treballar-hi aquests anys. La Júlia, que aquesta tesis és impensable sense ella, i que neix del seu treball. El Toni, que junt amb la Júlia va engegar el projecte que ha donat llum a aquesta tesis ja fa uns quants anys. Al QQ, per moltes coses, però crec que sobretot perquè he fet un molt bon amic. Al Lluís, per les seves idees, divagacions i confiança. Per intentar no oblidar-me d'altres persones que m'han ajudat molt, la Berta que encara trobo a faltar, era un gust treballar-hi; i més gent del lab, l'Audrey gràcies pel seu anglès, al Xavi per l'estadística i la Myriam amb qui vem compartir molts anàlisis.

Finalment no fugiré del tòpic i evidentment la meva família, a qui sempre he tingut a prop. Als meus pares, per tot. A la Sílvia i la Mireia, al Pau, la Maria, la Berta i la Clara. I al Sergi i al David també, clar. I a la Neus. Per estar amb mi tots aquests anys. No crec que es pugui posar en paraules tot el que m'aportes.

PREFACE

This thesis represents a distinctive approach to comprehending epilepsy, viewing it through the lens of a network-based disorder. Within this framework, a multitude of biomarkers have been unearthed, each bearing the potential to play a pivotal role in guiding and informing patient consultations. By delving deeply into the intricate web of neuronal interactions, with a specific emphasis on encompassing the entire expanse of the brain, this approach provides a rich tapestry of insights. These insights, in turn, hold the promise of furnishing us with a profound understanding of how epilepsy manifests and responds to surgical interventions.

In particular, the focus on large-scale, whole-brain dynamics opens a gateway to a trove of valuable information. It offers us the ability to unravel the complex interplay of neural elements that underlie this condition. Beyond mere seizure occurrence, these biomarkers shed light on a broader spectrum of outcomes—ranging from the attainment of seizure freedom to the intricate domains of cognitive functioning and emotional well-being.

This comprehensive perspective permits us to navigate the complexities of epilepsy with greater clarity. It allows us to anticipate, to a certain degree, how the condition might respond to surgical interventions. Will seizures be curtailed? Will cognitive functions witness enhancement or preservation? Will emotional equilibrium be restored? These are all inquiries that stand to benefit from the insights garnered through this network-oriented investigation.

In essence, this thesis doesn't merely view epilepsy as isolated events within the brain's neural circuitry. Instead, it perceives it as a dynamic network, where disruptions can reverberate through intricate pathways. By identifying and deciphering the biomarkers that mark these disruptions, we can offer patients and their caregivers a more nuanced understanding of what lies ahead. It empowers us to provide guidance rooted in a deeper appreciation of the interwoven threads of epilepsy's impact on brain function and the potential trajectories it might take following surgical interventions.

LIST OF ABREVIATIONS

ASD – Anti Seizure Drug

AUC – Area Under the Curve

CA – Cornu Ammonis

DAN – Dorsal Attention Network

DC – Degree Centrality

DMN – Default mode network

DnD – De Novo Depression

DTI – Diffusion Tensor Imaging

EEG – Electroencephalogram

EXE – Executive Network

FA – Fractional Anisotropy

FDR – False Discovery Rate

fMRI – functional Magnetic Resonance Imaging

FWE – Family Wise Error

HS – Hippocampal Sclerosis

ICA – Independent Component Analysis

ICV – total Intracranial Volume

ILAE – International League Against Epilepsy

MD – Mean diffusivity

NAcc – Nucleus Accumbens

nD – No Depression

PET – Positron Emission Tomography

RAVLT – Rey Auditory Verbal Learning Task

ROC - Receiver characteristic Operator Curves

ROI – Region of Interest

rs-fMRI – resting state functional Magnetic Resonance Imaging

RSN – Resting State Networks

SEEG – Stereo Electroencephalography

SF – Seizure Free

TLE – temporal lobe epilepsy

VNS – Vagal Nerve Stimulation

ABSTRACT

Epilepsy, as stipulated by the ILAE, is a neurological disease marked by and enduring condition that ensures the persistence of epileptic seizures. It is a very heterogenous disease, with a variety of etiologies, disease course and response to treatment.

This study delves into the potential improvement of TLE, one of the most prevalent forms of epilepsy, through surgical intervention, while also exploring the associated costs and implications. For patients with TLE whose seizures remain uncontrolled despite drug treatment, surgery can significantly reduce seizure frequency and, in many cases, even lead to a cure. However, surgery can come with risks, and some patients may not experience improvement after the procedure or may encounter cognitive and psychiatric complications. Predicting which patients will benefit the most from surgical intervention has been a challenging task. In recent years, adopting a perspective that views TLE as a condition affecting neuronal networks has yielded some advancements in our ability to predict surgical outcomes.

With the aim of predicting the response to surgery of patients with TLE at the individual level in the different domains (seizure recurrence cognitive impairment, motivational deficits), our methodology primarily encompassed the utilization of two advanced neuroimaging modalities: DTI and rs-fMRI. These techniques offer a non-invasive means to comprehensively gauge the structural and functional connectivity of the entire brain. Our data was sourced from a cohort of TLE patients who were deemed candidates for surgical intervention due to the non-efficacy of traditional pharmacological interventions. Initial data acquisition was done prior to undergo a temporal lobe resection. Afterwards, patients were monitored by neurologists, tracking of seizure recurrences during long-term follow-up, as well as assessments of cognitive and emotional outcomes. Additionally, a normative group of

voluntary participants underwent the same neuroimaging protocols, thus providing a comparative baseline.

In study 1 and 2 we focused on the outcome of surgery in terms of seizure recurrence. In the first study, we measured the microstructural integrity of the hippocampal subfields contralateral to resection side. Both hippocampi are known to participate in seizure generation, and we hypothesized that the degree of microstructural damage to the non-resected hippocampus could relate to seizure persistence after surgery. Our experiment revealed that patients with decreased microstructural integrity of the contralateral hippocampus were more prone to relapse after surgery.

In the second study, we evaluated if the functional connectivity of the whole temporal lobe could be a marker of seizure relapse after surgery. Previous research has pointed to a whole brain network readaptation in patients with drug-resistant TLE, showing increased local and decreased long-range connectivity surrounding the area of origin of the seizures. In our study, we calculated the DC among three regions of the to be resected temporal lobe. We found that a specific reorganization in temporal lobe connectivity, with increased functional connectivity in the temporal pole and the mesial temporal area related to better prognosis after surgery, in terms of curation of having seizures.

In Study 3 and 4 we aimed to study the risk of cognitive and mood disorders after resection of the temporal lobe. In the third study, we analyzed the connectivity of both hippocampi to main whole brain resting state networks involved in cognition. Centered on the connectivity pattern of both hippocampi with the resting-state networks (DMN, DAN & EXE) we found differences in the connectivity of these structures relating to verbal learning decline after surgery. Moreover, these differences were found along the longitudinal axis of both hippocampi, highlighting the differences of connectivity in this structure.

Our study revealed that a distinct connectivity pattern among the anterior and posterior regions of the hippocampi with the default mode network and the dorsal attention network could identify those patients who were at risk of verbal learning decline after surgery.

Finally, in the fourth study, we compared the patients who developed the novo mood disorders after surgery. Specifically investigating the functional connectivity of the NAcc and the to-be resected hippocampus, we found that patients with a decrease of connectivity between these structures were more prone to develop de novo mood disorders after a surgical resection of the mesial temporal structures.

Our investigation yielded the identification of a variety of potential biomarkers, supported by prior empirical evidence. Significantly, in line with the individual heterogeneity of TLE, we assessed these markers at the individual subject level, and prior to temporal resection. The result of our experiments shed light on the intricate relationships between the identified biomarkers and the post-surgery clinical outcomes. Importantly, the approach that we adopted, understanding epilepsy as a network related disease not only proved to be insightful but also generated a novel set of presurgical biomarkers key in TLE surgery. These markers hold significant promise for tailoring personalized guidance for individuals with TLE who are preparing to undergo surgical interventions.

RESUMEN

La epilepsia, según lo estipulado por la ILAE, es una enfermedad neurológica caracterizada por una condición duradera que asegura la persistencia de ataques epilépticos. Es una enfermedad muy heterogénea, con una variedad de etiologías, curso de la enfermedad y respuesta al tratamiento.

Este estudio se centra específicamente en la cirugía de la TLE, uno de los tipos más comunes de epilepsia: como el procedimiento quirúrgico puede mejorar la frecuencia de crisis, y con que posibles secuelas es una pregunta difícil de responder a nivel individual. Para los pacientes con TLE cuyas crisis permanecen sin control a pesar del tratamiento farmacológico, la cirugía puede reducir significativamente la frecuencia de las convulsiones y, en muchos casos, incluso conducir a una cura. Sin embargo, la cirugía puede conllevar riesgos, y algunos pacientes pueden no experimentar mejoría después del procedimiento o pueden encontrar complicaciones cognitivas y psiquiátricas. Predecir qué pacientes se beneficiarán más de la intervención quirúrgica es una tarea difícil. En los últimos años, la hipótesis de la TLE como una condición que afecta las redes neuronales ha producido algunos avances en nuestra capacidad para predecir los resultados quirúrgicos.

Con el objetivo de predecir la respuesta a la cirugía de pacientes con TLE a nivel individual en diferentes dominios (recurrencia de crisis, déficits cognitivos y atencionales), nuestra metodología abarcó principalmente la utilización de dos modalidades avanzadas de neuroimagen: DTI e imágenes de rs-fMRI. Estas técnicas ofrecen un medio no invasivo para medir la conectividad estructural y funcional de todo el cerebro. Nuestros datos se obtuvieron de una cohorte de pacientes con TLE que se consideraron candidatos para la intervención quirúrgica debido a la no respuesta a fármacos. La adquisición inicial de datos se realizó antes de someterse a una resección del lóbulo temporal. Posteriormente, los pacientes fueron monitoreados por neurólogos, evaluando la recurrencia de crisis durante el seguimiento

a largo plazo o la aparición de secuelas cognitivas y psiquiátricas. Además, un grupo normativo de participantes voluntarios se sometió a los mismos protocolos de neuroimagen, proporcionando así una línea de base comparativa.

En los estudios 1 y 2 nos centramos en el resultado de la cirugía en cuanto a la recurrencia de las crisis. En el primer estudio, medimos la integridad de los subcampos del hipocampo contralaterales al lado de la resección. Se sabe que ambos hipocampos participan en la generación de las crisis, y planteamos la hipótesis de que el grado de daño microestructural al hipocampo no resecado podría relacionarse con la persistencia de estas después de la cirugía. Nuestro experimento reveló que los pacientes con una pérdida de la integridad microestructural del hipocampo contralateral eran más propensos a la recaída después de la cirugía.

En el segundo experimento, evaluamos si la conectividad funcional de todo el lóbulo temporal podría ser un marcador de recaída de las crisis tras la cirugía. Investigaciones previas en pacientes con TLE fármaco-resistente apuntan a una readaptación de toda la red cerebral, con un aumento de la conectividad local y una disminución de la conectividad de largo alcance que rodea el área de origen de las crisis. En nuestro estudio, calculamos el DC de tres regiones del lóbulo temporal a resecar. Encontramos una reorganización de la conectividad del lóbulo temporal, destacando que el incremento de la misma en el polo y en la región temporal mesial se relacionó con un mejor pronóstico después de la cirugía.

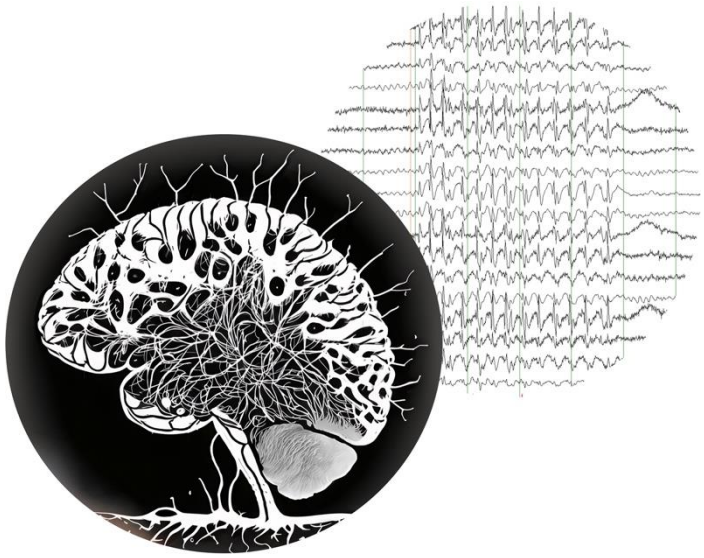
En los estudios 3 y 4 nuestro objetivo fue estudiar la posibilidad de trastornos cognitivos y del estado de ánimo después de la resección del lóbulo temporal. En el tercer estudio, analizamos la conectividad de ambos hipocampos con las redes de estado de reposo del cerebro involucradas en la cognición. Este estudio se centró en la conectividad entre los

dos hipocampos y las llamadas redes cerebrales de reposo (DMN, DAN & EXE), encontrando diferencias entre la conectividad de estas estructurales y una caída en la capacidad de aprendizaje verbal tras la cirugía. Estas diferencias las hallamos a lo largo del eje longitudinal del hipocampo, destacando por tanto la importancia en las diferencias de conectividad dentro del propio hipocampo. Nuestro estudio reveló que un patrón concreto de conectividad entre ambos hipocampos y las redes de cerebrales de reposo permitía identificar aquellos pacientes con un riesgo elevado de caída de aprendizaje verbal tras la cirugía.

Finalmente, en el cuarto estudio, comparamos los pacientes que desarrollaron depresión después de la cirugía. Al investigar específicamente la conectividad funcional del NAcc y el hipocampo resecado, encontramos que los pacientes con una disminución de la conectividad entre estas estructuras eran más propensos a desarrollar trastornos del estado de ánimo *de novo* después de una resección quirúrgica de las estructuras temporales mesiales.

Nuestra investigación produjo la identificación de una variedad de potenciales biomarcadores, respaldados por evidencia empírica previa. Significativamente, en línea con la heterogeneidad individual de la TLE, evaluamos estos marcadores a nivel de individual antes de la resección temporal. El resultado de nuestros experimentos arrojó luz sobre las intrincadas relaciones entre los biomarcadores identificados y los resultados observados. Es importante destacar que el enfoque centrado en entender la epilepsia como una enfermedad de redes cerebrales que adoptamos no solo demostró ser útil, sino que también generó un nuevo conjunto de marcadores. Estos marcadores son prometedores para adaptar la orientación personalizada para las personas con TLE que se están preparando para someterse a intervenciones quirúrgicas.

INTRODUCTION



1 INTRODUCTION

1.1 What is epilepsy?

Epilepsy is a disease of the brain, as defined by the ILAE, characterized by an enduring predisposition to generate epileptic seizures. Epilepsy is one of the most prevalent neurologic conditions, affecting approximately 70 million people worldwide (Sander et al., 1996). The yearly incidence is estimated at around 50 new cases per 100000 individuals, though this rate varies between 40 to 70 cases per 100000. (Thijs et al., 2019).

There are many diseases that can cause epilepsy; also, there is great heterogenicity in the individual form of clinical presentation and response to treatment among patients. Identifying the source of this clinical and therapeutical variability is crucial and relates to the understanding of the pathogenesis of epilepsy. Besides, it is important to note that beyond the seizures, epilepsy encompasses a high range of cognitive and motivational alterations that can result in psychological and social consequences impacting patients function and quality of life equally, or sometimes even more significantly, than seizures (Fisher et al., 2014).

As highlighted, epilepsy manifests as a remarkably heterogeneous condition, exhibiting considerable variation among individual patients. The consequences of structural and functional alterations within the pathological substrate of epilepsy, coupled with the ongoing experience of seizures, give rise to a myriad of neurological deficits. Given the diversity in the pathological substrate and the frequency and severity of seizures across patients, it is imperative to prioritize the development of personalized biomarkers capable of characterizing distinct clinical profiles. These individualized markers would not only enhance our understanding of the disease but also pave the way for more targeted and effective therapeutic interventions. The treatment aims to achieve seizure freedom, which significantly

reduces the psychological, cognitive and social burden experienced by patients with epilepsy (Devinsky, 1999).

1.1.1 Epilepsy: definitions and classification

Being an heterogenous disease, with many etiological entities associated to cause epilepsy, important efforts have been directed towards its classification (Berg et al., 2010). The classification of epilepsy is a key clinical tool in the evaluation of a patient with seizures, and serves many purposes from treatment to basic research (Scheffer et al., 2017).

Among the diverse classifications of epilepsy, one of the first steps involves distinguishing between patients who suffer by either focal or generalized seizure types. Despite they can coexist in a limited number of epilepsy patients, most of them exhibit one type of seizure, either generalized or focal. Focal seizures are by definition originated in neuronal networks limited to one hemisphere, more or less distributed, but ictal onset is consistent from one seizure to another (Berg et al., 2010). In contrast, generalized seizures originate in widely distributed networks spanning both hemispheres, and the onset region is not always consistent from one seizure to another (Berg et al., 2010). Definition of the seizures as focal or generalized is determined foremost by clinical findings during the seizures, together with EEG recordings when possible and, in some cases, also with imaging findings (Berg et al., 2010).

Once the predominant seizure type of a patient is identified, the classification of epilepsy can be done accordingly. Patients with focal seizures are classified as having focal epilepsy. Similarly, patients with generalized seizures are grouped under generalized epilepsy. For patients who exhibit both type of seizures, their classification is denoted as combined generalized and focal epilepsy (Scheffer et al., 2017).

Furthermore, within this classification scheme, there are characteristic Epilepsy Syndromes, which are defined as a cluster of features including seizure types, EEG signatures, imaging findings and neuropsychological profiles. (See Figure 1).

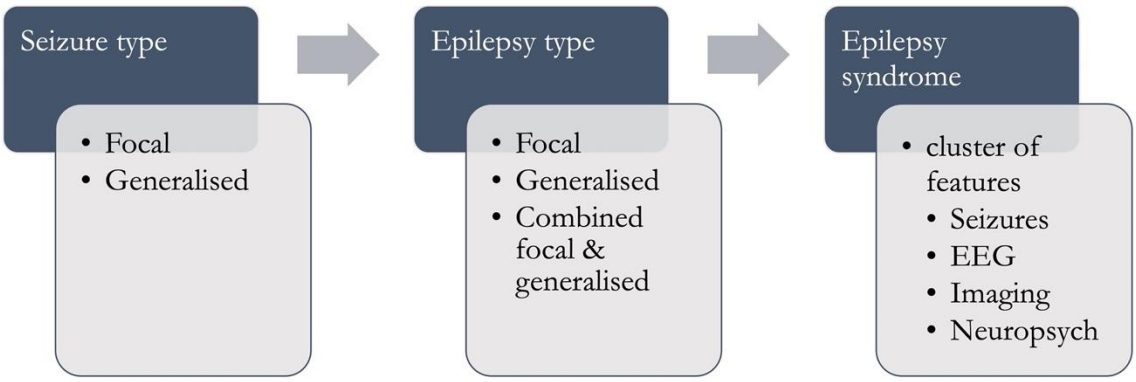


Figure 1. Schematic representation of the classification of seizures, epilepsy types and epilepsy syndromes.

The current classification of the ILAE recognizes multiple epilepsy syndromes, such as childhood absence epilepsy or Dravet syndrome. For instance, in the case of focal epilepsy, a classical syndrome is TLE-HS. (See Figure 2). This syndrome typically arises when focal seizures originate in the temporal lobe, more specifically its mesial regions. This area is one of the most frequent areas of onset, and is crucial for sustaining the focal seizure network (Tatum, 2012).

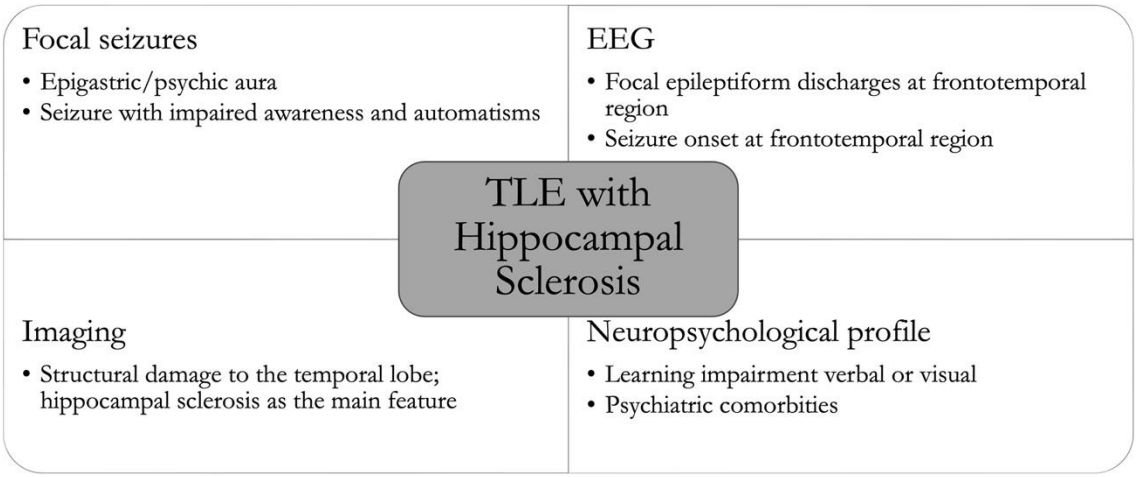


Figure 2. Example of a classical epileptic syndrome due to a stablished etiology. TLE-HS consists of a plethora of clinical, EEG, imaging and neuropsychological findings, that can be identified among different patients and be caused by different etiologies.

Finally, once the specific seizure type and classification of epilepsy has been defined, the next step involves identifying the underlying etiology of the epilepsy. Currently, etiological factors are categorized into distinct groups, including structural causes (involving brain lesions), genetic predisposition, infectious origins, metabolic triggers, or immune-related causes. There remains a subset of patients for whom a definite etiological cause cannot be determined, resulting in the classification of epilepsy as having an unknown origin. It is important to note that different etiologies may end up in the manifestation of the same epilepsy syndrome (Scheffer et al., 2017).

To further understand the afore-mentioned concepts of focal and generalized, which apply to seizure and epilepsy, and also how the relation with neuronal networks have been established, it is necessary to look the history of clinical description of seizures and epilepsy, and how new insights shaped the already stablished conceptualizations.

1.1.2 Historical perspective

The earliest description of seizures can be traced back to Sumerian documents dating around 2500 BC in Mesopotamia. The ancient text depicts a person with a twisted neck, with extremities tense, eyes open, frothing at the mouth, and a loss of consciousness. Moreover, an ancient Babylonian tablet dated around 1000 BC contains descriptions of what we now identify as focal onset, tonic, and absence seizures, as well as descriptions of prodromal symptoms including, auras, postictal phenomenon, interictal emotional disturbances, and seizure precipitants (Patel et al., 2020).

Despite accurate description of the symptoms of seizures, the explanations for seizures and epilepsy during this era were entrenched in magical thinking and supernatural beliefs. It was during the time of Hippocrates (around 400BC) that a scientific explanation for epilepsy or seizures was first proposed. *On the Sacred Disease*, a book within the Hippocratic collection of medical writings, represents the first attempt to view epilepsy in a scientific

and rationale way. Authored by an unidentified physician, this work makes a significant shift, by stating that the seat of the disease lied in the brain, an organ of senses, motion and intellect. From the Greek language come the words "seizure" (to take hold), "aura" (breeze) and even "epilepsy", which is derived from the Greek word "epilambanein", which means "to be seized." (Patel et al., 2020).

The contemporary terms and definitions related to seizures, epilepsy and epileptic syndrome were mainly developed during the 19th and 20th century. Changing perceptions of epilepsy, made patients suffering from the disease to be segregated from criminals and the mentally ill in asylums. This shift gave rise to the development of dedicated colonies and hospitals in western Europe and America, designed to provide specialized care for these individuals. This approach enabled clinicians to closely observe patients and introduce a new lexicon to describe seizures (Patel et al., 2020).

Together with the advent of EEG in the early 20th century, the understanding of epilepsy profoundly increased, which was accompanied by more accurate clinical descriptions. The concepts of "focal" and "generalized" seizures and epilepsies were progressively stablished, by the finding of two fundamentally different types of epileptiform EEG patterns observed (Wolf, 2014) (See Figure 3). During the first half of the 20th century, the notion that epilepsy might result from hypersynchronization of specific neuronal clusters or brain structures also emerged. This hypothesis, based on EEG recordings and tied to the results of epilepsy surgeries, represented a significant advancement (Penfield et al., 1954).

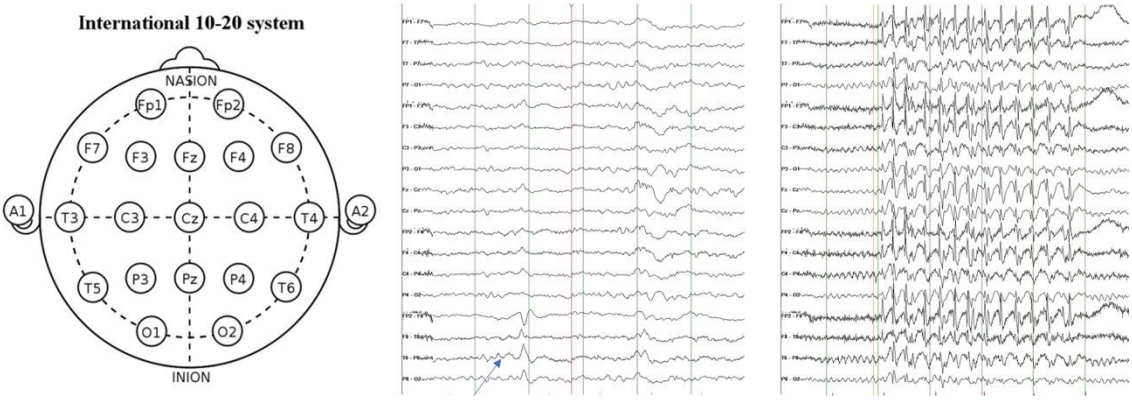


Figure 3. The advent of EEG deeply modified the understanding and classification of epilepsy. On the left, the classical disposition of scalp EEG electrodes, based on international convention (the 10-20 system). To the right, the two most typical EEG patterns in epilepsy; first, a focal discharge (arrow) over the right fronto-temporal electrodes (F8-T8). Next, a generalized discharge, visible along the whole electrode set. These patterns contributed to the concepts of neuronal synchronization, and the dichotomy between focal and generalized.

The International League Against Epilepsy (ILAE), an international organization established in 1909, introduced for the first time a classification system of seizures in 1964, led by Professor Henri Gastaut. This system encompassed seizure-type categories such as partial, generalized, unilateral or predominantly unilateral in children, erratic in newborn, and unclassified seizures. Notably, Gastaut acknowledged the interchangeability of terms like partial, focal, and local. However, he favored the term "partial" due to its historical prevalence and most widely usage at the time. He believed that this term more accurately depicted the discharging hypersynchronous neuronal population throughout a brain region. This population was believed to be widely located throughout a region of the brain, and therefore, he argue, couldn't be properly described as focal or local (Patel et al., 2020).

This classification system was further revisited and expanded, evolving to encompass not only seizures but also distinct types of epilepsies based on the predominant type of seizure. This evolution occurred in 1981 and again in 2001. Employing a dual dichotomy approach, the first divided epilepsy by semiology into generalized or localization-related partial or focal, while the second divided epilepsy by etiology into symptomatic or "secondary", idiopathic (primary) or cryptogenic causes (Engel Jr., 2001; Jerome Engel, 2006).

In 2010, the definition and classification mentioned earlier was published, marking a significant milestone (Berg et al., 2010). Noteworthy, it is in this last classification that the novel concept of neuronal network is introduced. For the first time, it delineates that the generalized seizures originate at some point within, and rapidly engage bilaterally distributed networks, whereas focal seizures originate within networks confined to one hemisphere. Subsequently, the concept of an electroclinical syndrome was established. This term, "syndrome", was reserved for a set of clinical entities reliably identified by a cluster of electroclinical characteristics (Scheffer et al., 2017).

As mentioned, along the years the concept of focal or generalized neuronal generators of seizures due to underlying neuronal hypersynchronous discharges had been included in the classification schemes; initially dichotomized in focal and generalized. This was challenged in 2002, with the definition of identifiable neuronal networks comprising more or less extended neuronal structures underlying any type of seizures and epilepsy (Spencer, 2002). In 2012, new insights into ictogenesis led to the hypothesis of "system epilepsies," postulating that the propensity to generate seizures of some epilepsies is due to the susceptibility of an identifiable neural system, specifically its inner interactions, and can be made up of more or less distant brain areas. This approach goes beyond the simple dichotomy between focal and generalized epilepsy (Avanzini et al., 2012).

The idea of epilepsy being a network or system-level neuronal disease differs from the concept of epilepsy originating from the sequential propagation of hypersynchronous discharge, covering a relative small (focal) or wider (generalized) area. Instead, results from research at the turn of the 21st century have revealed much more complex neuronal dynamics, focusing the origin of seizures and epilepsy to the interplay among sets of functionally and anatomically connected neuronal populations spanning cortical and subcortical brain structures. In this framework, activity in any given area affects activity in all the others, and

the interaction among sets of neurons is crucial in the generation of seizures and their comorbidities (Bartolomei et al., 2017; Nair et al., 2004; Spencer, 2002; Warren et al., 2019).

1.1.3 From hypersynchrony to complex dynamics: The network concept

The ILAE currently define an epileptic seizure as a transient event marked by signs and/or symptoms arising from an abnormally excessive or synchronized neuronal activity within the brain. This clinical incident encompasses a wide range of potential manifestations. This definition includes both the substrate of a seizure's occurrence (abnormal excessive or synchronous neuronal activity) and its origin (the brain) (Fisher et al., 2014).

Along history, several theories regarding the causes and origins of seizures have been proposed. As mentioned earlier, Hippocrates was the first author in attributing seizures to the brain, yet this view was not accepted for many years. It wasn't until the end of the 19th century that this idea was well stablished (Patel et al., 2020). The emergence of the EEG technique saw more advances, especially in understanding seizures as originating from different parts of the brain and translating into different symptoms accordingly (Wolf, 2014). Alongside advances in neuroimaging techniques emerge the definite relation between origin of seizures in some patients and the presence of brain lesions (Patel et al., 2020).

Since the 19th century, and especially after the advent of EEG, epilepsy's hallmark was abnormally enhanced neuronal excitability and synchronization. Indeed, the term hypersynchrony was coined to describe the distinctive high-amplitude pathological neuronal behavior (Fisher et al., 2014; Penfield et al., 1954). However, it has become more evident that it is not only the behavior of individual components within the brain, but also the interactions among brain regions, involving hundreds or thousands of neurons organized into neuronal networks, that give rise to neural functional states (Lynn et al., 2019).

Seizures, and therefore epilepsy, must be understood as one of several possible brain states, supported by an underlying neuronal network (Jirsa et al., 2014). Seizures can manifest under diverse conditions, even within so-called 'normal' networks, can be induced across species and triggered by a range of different lesions while remaining consistent regarding neurophysiological signatures; all this facts suggest that they belong to the dynamic repertoire of neural activities (Jirsa et al., 2014). Neurophysiological studies of functional coupling within networks underlying complex ictal behavior indicate that the clinical semiology of a given seizure depends upon neither the anatomical origin of ictal discharge nor the target areas of its propagation alone but on the dynamic interaction between these areas (Chauvel et al., 2014).

In this sense, epileptiform phenomena, particularly seizures, result from complex interactions within sets of neuronal networks characterized by the underlying heterogeneity of microscale neuronal firing and dynamical evolution of synchronization (Jiruska et al., 2013). Initially, the seizure state was considered as being predominantly composed of strong hypersynchronous functional connections (Penfield et al., 1954). In contrast, a significant body of recent research presents compelling evidence that complex changes among both strong (synchronized) and weak (desynchronized) network nodes accompany seizure dynamics (Szabo et al., 2015). These changes exhibit varying degree of spatial extension, with seizures potentially originating from multiple distributed cortical microdomains (Feldt Muldoon et al., 2013a). Consequently, these events can affect cortical regions inside and outside the seizure focus: the neural dynamics that generate an epileptic network are characterized by pathologic, seizure-generating 'foci' embedded in a web of structural and functional connections (Jiruska et al., 2013).

As this web of connections presents with high individual variability, it entails for the great clinical heterogenicity of seizures, the variability in response to certain treatments and

the comorbidities that patients suffer (Bartolomei et al., 2017; Hal Blumenfeld et al., 2004; Spencer, 2002). This variability is particularly pronounced in focal epilepsy, a condition traditionally viewed as a localized brain disorder. However, evidence has demonstrated widespread network alterations extending beyond the epileptogenic zone from which seizures originate (Englot et al., 2016). (See Figure 4)

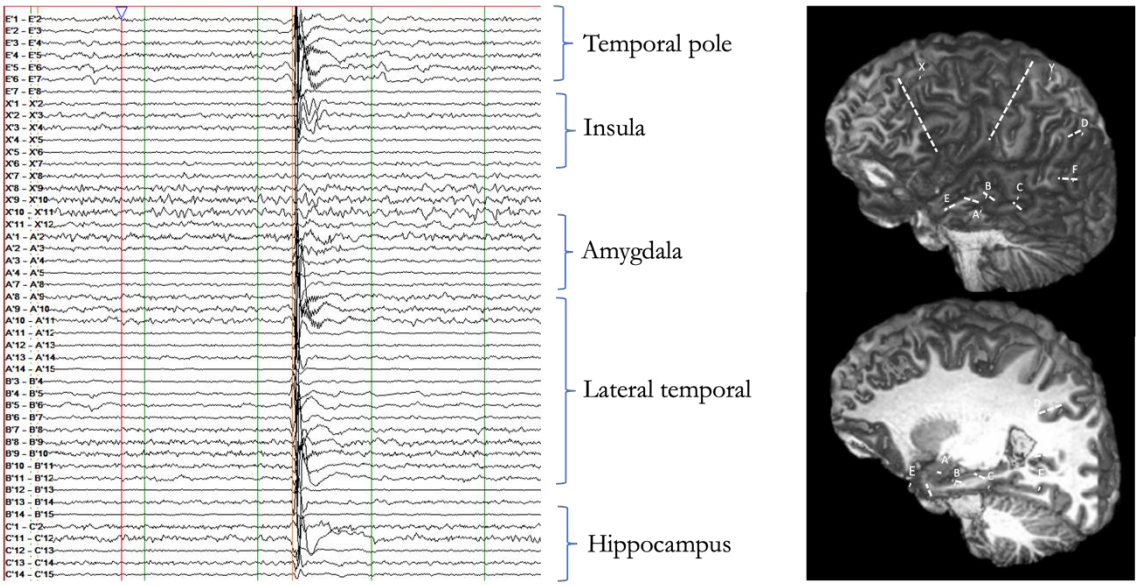


Figure 4. One of the techniques that proved useful in the study of epilepsy networks is intracranial EEG. On the left, an interictal spike in the center of the image; it can be seen in a large number of intracranial electrodes, which are sampling far apart structures as the temporal pole, de insula, the mesial temporal area and the lateral temporal wall. On the right, an schematic vision of the trajectories of the intracranial electrodes. The patient had temporal lobe epilepsy with hippocampal sclerosis; the involvement of this set of structures is typical of this syndrome, and depending on the implication as parts of the network of this set of structures surgery may or may not be a solution (Carmen Barba et al., 2016)

The configuration of brain networks can be assessed and quantified through a diverse array of techniques. These methodologies offer valuable insights into the intricate connections and interactions within the brain's intricate web of neurons. In the forthcoming sections, I will delve into a comprehensive exploration of these techniques and how they contribute to unraveling the complexities of normal and epileptic brain states, offering potential avenues for diagnosis, treatment, and enhanced neurological insights.

1.2 Epilepsy treatment

The history of epilepsy treatment in the Western world, as with the history of epilepsy itself, spans over 4 millennia, with attesting documents dating back to ancient civilizations in the Middle East. Throughout the ages, treatments for epilepsy have been predominantly based on empirical methodologies, often influenced by prevailing beliefs in medicine, theology, or superstitions. Ancient physicians relied on detailed clinical observations to distinguish different types of epileptic syndromes and to speculate about their potential origins (Patel et al., 2020).

As previously mentioned, early pathophysiological theories correctly pinpointed the brain as the source of the disorder, but they often attributed incorrect causes. Therapeutic methods included prescribed diets, living conditions, occasional surgeries such as bloodletting or skull trepanation, and the use of medicinal herbs. Despite these treatments were rooted in pathophysiological principles, they were frequently ineffective compared to the more empirical therapies of modern times. Throughout history, epilepsy has unfortunately been associated with occult or evil influences, which gained adherents even within the medical community.

In the late 19th and early 20th century, the concept that seizures could arise from focal hypersynchrony emerged in fact from both clinical and experimental research, leading to the successful control of seizures using early sedative drugs like bromides and barbiturates. The introduction of phenytoin in the 1940s demonstrated that non-sedative drugs could also effectively control seizures. Additionally, the development of in vivo seizure models expanded the range of pharmaceutical agents tested for their efficacy against epilepsy (R. A. Gross, 1992).

In parallel to drug development, epilepsy surgery has proven to be an effective treatment for seizures and epilepsy since the late 19th century. Before the advent of the EEG

technology, surgical interventions were limited to patients whose seizures originating near primary neocortical areas or those with visible structural lesions. However, significant advancements in electrophysiological mapping of epileptogenic brain tissue in the 1940s and 1950s led to increased interest in surgical treatment, especially for TLE. Over the following decades, multiple epilepsy surgery centers emerged worldwide, each adopting various approaches (J. Engel, 1992).

1.2.1 Antiseizure drugs

Currently, the first step in the treatment of epilepsy is always pharmacological; ASD are the main treatment modality, and the aim is always to try to reach seizure freedom without significant side-effects that can affect the quality of life. Reaching seizure remission translates in decreased morbidity and might also reduce premature mortality associated with convulsive seizures (Devinsky et al., 2015).

Currently, there exist over 25 approved medications for managing seizures (Thijs et al., 2019). The symptomatic relief of seizures with antiseizure drugs occurs through their interactions with a variety of cellular targets, which can be classified into several categories: modulation of voltage-gated ion channels, including sodium, calcium, and potassium channels; enhancement of GABA-mediated inhibition; direct modulation of synaptic release through effects on components of the release machinery; and inhibition of synaptic excitation mediated by ionotropic glutamate (Löscher et al., 2010). These actions reduce the probability of seizure occurrence by modifying the neural bursting properties and diminishing synchronization within localized neuronal ensembles. In addition, ASDs inhibit the propagation of abnormal firing to both adjacent and distant brain regions (Löscher et al., 2020).

Noteworthy, ASD are designed to control seizures, and do not address the underlying causes of epilepsy. Therefore, they need chronically use. It is important to note that up to 40% of patients fail to achieve seizure freedom despite ASDs treatment (Kwan et al., 2000).

These patients, who do not respond to drug treatment, face lifelong persistent seizures (Kwan et al., 2010), leading to the term "drug-resistant epilepsy" being used to characterize their chronic condition.

Different patterns of seizure relapse during life has been described, and alternating periods with different seizure frequencies may occur (Löscher et al., 2020). However, long-term outcome studies show that when seizure control fail with two well tolerated anti-seizure drugs, the chances of effective seizure management through further drug trials become poor (Chen et al., 2018; Kwan et al., 2010). There are several mechanisms of drug resistance reported, but no methods for overcoming any of these mechanisms have yet been implemented (Löscher et al., 2020).

Patients affected with drug-resistant epilepsy have considerable socioeconomic and psychological limitations that decrease their quality of life and functionality, increasing their risk of mortality (Kalilani et al., 2018). It is in this situation of drug-resistance when the demand of advanced therapies is needed. One of the most useful therapies is brain surgery, offering promise for selected patients with focal seizures that have been proven to be resistant to ASD.

1.2.2 Epilepsy surgery

As mentioned, surgery has the potential to cure epilepsy (De Tisi et al., 2011), however, that is not always the result. In patients with focal epilepsy, it is important for the outcome of surgery to correctly localize and remove the area responsible of seizure generation within the brain, the so-called epileptogenic network (Bartolomei et al., 2017). One of the most frequent localizations, as previously stated, is within the temporal lobe (De Tisi et al., 2011). The use of epilepsy surgery goes back to the late 19th century; as such, along these years, an evolution of different approaches and considerations about the area needed to be resected for the best outcome has been seen.

The practice of surgery begins as early as 1879, when William Macewen successfully localized and resected a frontal meningioma based on the semiology of the focal motor seizures. Noteworthy is the work of Hughlings Jackson. In describing cases of syphilitic epilepsy, his observations centered around unilateral convulsions, and his anatomical investigations showed the cause was obvious organic disease on the side of the brain opposite to the side of the body that convulsed. Jackson's collaboration with surgeon Victor Horsley has been said to have marked the birth of epilepsy surgery, the relationship between epilepsy and a structural cause and the closely related term "focal epilepsy". Both Horsley and Jackson, have been credited for successfully localize and remove epileptogenic lesions in three patients with partial seizures at the London's National Hospital in 1886 (J. Engel, 1992). A patient of theirs was operated on based on the anatomical conclusion from seizure semiology; Horsley removed a tuberculoma from a region of the cortex, which he and Jackson considered the "epileptogenic focus," and the patient became seizure-free (SF). Afterwards, in the 1950s, Penfield and Herbert Jasper introduced intracranial EEG as a routine method in neurosurgery, and conceptualized the "epileptogenic zone", based on intracranial EEG recordings as the area needed to be resected in order to render a patient seizure free (Penfield, 1956).

In the last half of the century, the rise of neuroimaging further supported Jackson's structural theory of epilepsy, and the localization of structural lesions came closely related with surgery. The invention of computerized tomography first and later MRI made a great impact, allowing for the identification of even subtle brain lesions. Further techniques including fMRI, positron emission tomography, single-photon emission computed tomography, and magnetoencephalography continued to make significant contributions to the detection of epileptogenic lesions. Along with this came the rise of epilepsy surgery and the

use of modern techniques of intracranial EEG and SEEG to precisely localize the epileptogenic zone (Patel et al., 2020).

The relation between the cause of seizures (e.g. a structural lesion) and the "epileptogenic zone" has produced a long debate in the epilepsy community, as it is not always straight forward; further, the approach of seizures as a network disease has changed paradigms on how to correctly identify the area needed to be resected (Jehi, 2018). It must be noted that the concept of seizures arising from neuronal networks has had a close, bidirectional relationship with the evolution of surgery, in both surgery influencing the development of the concept and the network frame influencing surgical techniques.

The goal of this thesis is to amalgamate the network concept and the measures derived from this framework into distinct markers for predicting surgical outcomes in one of the most common epilepsy surgery procedures: temporal lobe resection for TLE.

1.2.3 Surgery and the network concept

The current concept of epileptogenic zone and network has evolved along time, as different frameworks have been used to explain the generation and propagation of seizures and the surgical approach (Nair et al., 2004). As mentioned earlier, initial works by Penfield and Jasper conceptualized the structures underlying seizure generation and needed to be resected to reach successful surgery in a more regional approach, the so-called epileptogenic zone, mostly restrained by the initial ictal-onset zones as defined by neurophysiologic recordings (Penfield, 1956). Afterwards, Bancaud and Talairach expanded the area, to include an "early propagation zone", based on the propagation of seizures to different areas, also based on neurophysiological recordings (Talairach et al., 1992). More recently, Susan Spencer developed the concept of large networks underlying seizures, compelling evidence from neurophysiologic and neuroimaging findings in patients with focal epilepsy (Spencer, 2002).

Noteworthy, this evolution of concepts for the epileptogenic network has been in parallel to the development of different techniques of intracranial EEG; different approaches to implantation of intracranial electrodes have provided different insights in the understanding of the underlying neural dynamics of seizures. (See Figure 5).

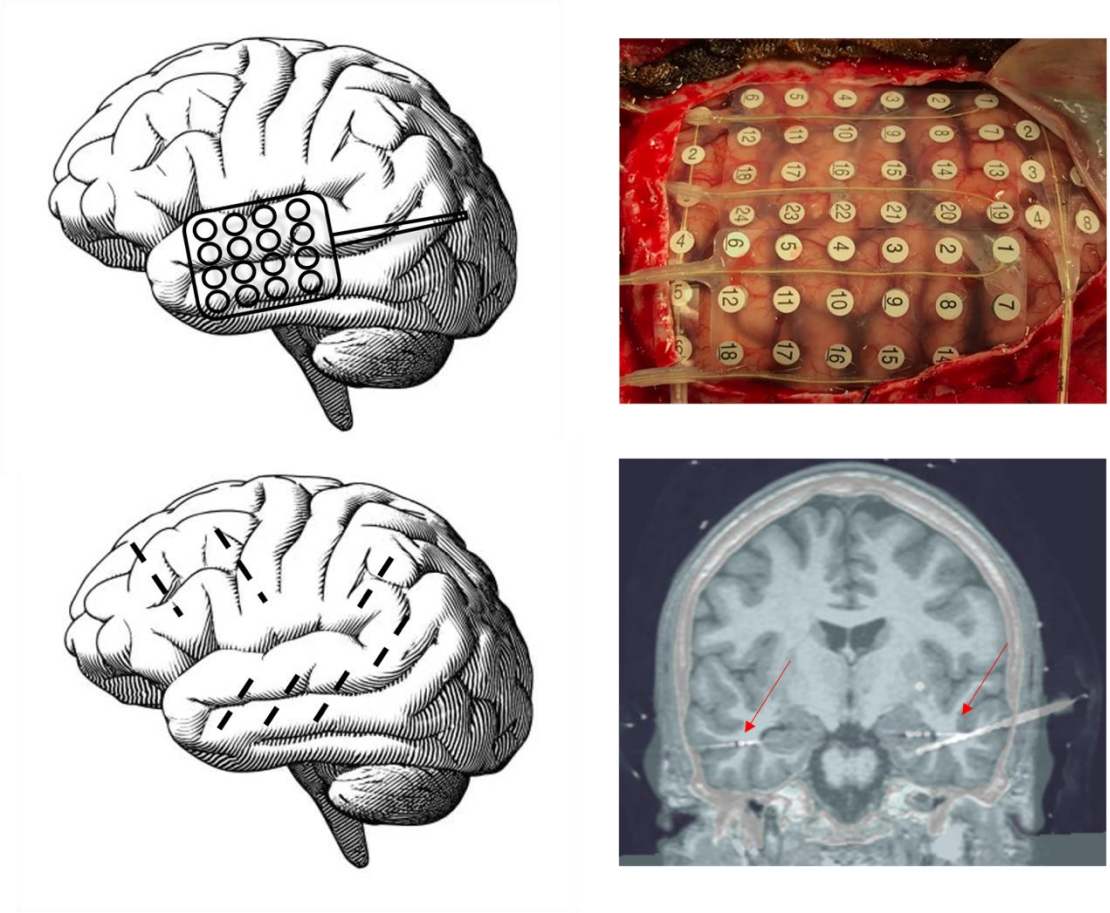


Figure 5. Two different techniques of intracranial EEG. On top, a so-called subdural "grid" of electrodes; on the left a schematic view, to the right a real-life picture of an implantation. Subdural grids were used by Penfield and Jasper and were very useful to localize seizures on the surface of the cortex; it partly lead to the epileptogenic zone hypothesis. Below, an SEEG schematic view, and on the left a real-life implantation, in this case an MRI image showing the SEEG electrodes (red arrows). SEEG provides a different approach, as the surgeon can implant electrodes along a wide area, and helped developing the network concept, as it is able to record far distant sites, even bilaterally.

A network is conceptualized as a functionally and anatomically connected, bilaterally represented, set of cortical and subcortical brain structures and regions in which activity in any one part affects activity in all the others. The essential operational component of this definition is the observation that vulnerability to seizure activity in any one part of the network is influenced by activity everywhere else in the network, and that the network as a

whole is responsible for the clinical and electrographic phenomena of human seizures. The interruption of the network, in a structural sense, or modification of network activity by electrical, biochemical, or metabolic influences in any part of the network will alter seizure expression or its occurrence (Spencer, 2002). See Figure 6

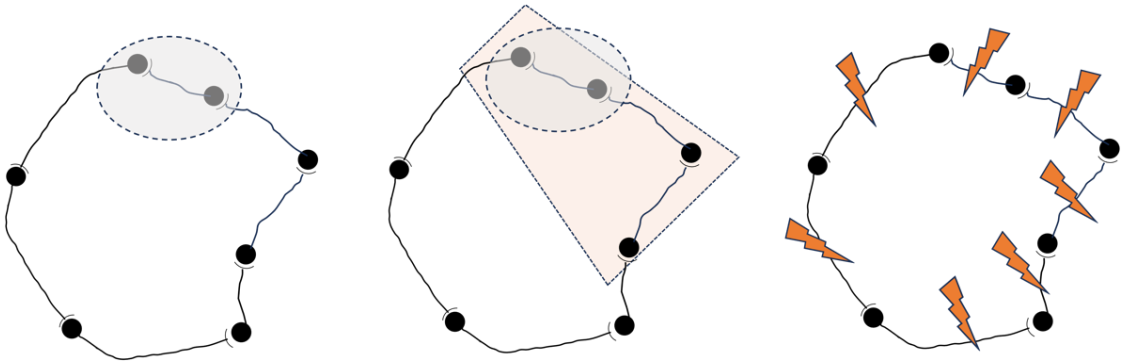


Figure 6. Diagrams representing the different concepts in epilepsy surgery, adapted from (Nair et al., 2004). First to the left, Penfield/Jasper concept of epileptogenic zone Seizure-freedom is achieved by resection of the actual seizure-onset zone; "early" seizure spread zone is not part of the epileptogenic zone. Second, in the middle, Tailarach/Bancaud concept of epileptogenic zone, figure illustrates the seizure-onset zones (darkly shaded area) and the "early" seizure-spread zone (lightly shaded area): the Tailarach/Bancaud hypothesis suggests that seizure-freedom requires resection of the seizure-onset zone and "early" seizure-spread zone. Finally, on the right, the concept of Large Network Hypothesis: all parts of the neuronal network are equally important for the generation of seizures. Seizure-freedom can be achieved by interruption of the network at any level (noted by a orange lightning).

Evidence of network disruption related to improvement of epilepsy is abundant. From several years it has been reported that lesions distant from the epileptogenic focus or the resection of brain areas even though with no pathological evidence of neuronal damage can improve seizures (Jehi, 2018). Further, several other neurosurgical methods beyond resection, such as VNS, also function in this framework. VNS modifies whole brain connectivity: by stimulating the peripheral vagus nerve, via its connections with the brainstem nuclei, it finally modifies thalamo-cortical pathways (Ibrahim et al., 2017). VNS has been proven in clinical trial as an effective treatment in all types of refractory epilepsy (Morris et al., 2013).

Hence, it holds significance to pinpoint network biomarkers that could potentially enhance the guidance offered to patients, both in terms of predicting seizure amelioration and mitigating post-surgical sequelae.

1.3 Temporal lobe epilepsy

TLE is one of the most prevalent types of focal epilepsy, and it is by far the most common type of drug-resistant epilepsy among patients considered for epilepsy surgery. Its incidence is approximately 0.51–0.66 cases per 1000 individuals, and it is estimated that in the United States, there are 3.1–3.4 cases per 100,000 people per year with drug-resistant TLE (Asadi-Pooya et al., 2017).

Is important to underline that a strict definition of TLE is lacking. It includes a heterogeneous range of disorders that comprises many different entities, all sharing the commonality of seizure origin within the temporal lobe. Therefore, surgical resection of either the entire lobe or a part of it may control the occurrence of seizures. Notably, temporal lobe surgery has been established as one of the more secure and most effective procedures for managing drug-resistant epilepsy (De Tisi et al., 2011).

The origins of this heterogeneity must be sought within the diverse range of epilepsy causes that can give rise to similar yet distinct individual disease patterns, spanning from genetic to structural factors, and even a substantial number of cases with an unknown origin (Bonilha, Martz, et al., 2012; Malmgren et al., 2012; Tatum, 2012). Together with this, the complex behavior of neuronal pathologic epileptic tissue, from the microscale domain (Szabo et al., 2015) to the broader "macroscale" evidence (Spencer, 2002) has demonstrated significant interindividual variability, resulting in a spectrum of clinical, cognitive, and psychiatric symptoms.

Given this circumstance, the heterogeneity of the disease underscores the significance of identifying variations in clinical, neuropsychological, and psychiatric profiles that may facilitate more tailored treatments (Ryvlin et al., 2016). In this regard, the discovery of biomarkers capable of enhancing the profiling of these variations could prove instrumental as a clinical tool for improving our management of these patients. This is particularly salient

in the context of surgical interventions, as this variability can be challenging to identify accurately before initiating treatment, and where a range of outcomes encompassing seizures, cognitive function, and mood have been documented (Bonilha, Martz, et al., 2012).

For a better understanding of this subject, we will first review the anatomical aspects of the temporal lobe, then it's connectivity and finally its relationship with surgery.

1.3.1 Anatomy and connectivity of the temporal lobe

Approximately 17% of the volume of the human cerebral cortex, 16% in the right and 17% in the left hemisphere, forms the surfaces of the temporal lobes. Temporal cortex includes areas involved with the auditory, olfactory, vestibular, and visual senses, and in the perception of spoken and written language. In addition to the superficial cortex, the temporal lobe contains white matter, part of the lateral ventricle, the tail of the caudate nucleus, the hippocampal formation, and the amygdala. The medial side of the temporal lobe includes regions concerned with olfaction (the uncus and nearby cortex) and memory (the hippocampal formation). (Kiernan, 2012).

One of the most relevant parts of the temporal lobe for its role in seizures and TLE is the hippocampal formation. The components of the hippocampal formation are the hippocampal complex, an enrolled gyrus with a three-layered allocortex with lamellar organization adjacent to the parahippocampal gyrus, the dentate gyrus and the associated white matter, the alveus, fimbria, and fornix. The cortex adjacent to the hippocampus is known as the entorhinal area; it is present along the whole length of the parahippocampal gyrus (Tatum, 2012). There is a transitional zone between the entorhinal and hippocampal cortices, called the subiculum. The hippocampal formation has indirect afferent connections from the whole of the cerebral cortex, funneled through the adjacent temporal cortex and the subiculum. Its position on the edge of the temporal lobe cortex, together with other structures at the edge of brain cortex as the cingulate gyrus form a ring, often called the

limbic lobe or limbic system (from Latin limbus, a hem or fringe), a name bestowed by Broca in 1877 (Kiernan, 2012).

The hippocampal complex, further divided into the head, body, and tail, spans approximately 4 to 4.5 cm. Within it, the Cornu Ammonis (CA1-CA4) subfields are enclosed by the dentate gyrus and exhibit extensive anatomical and functional connections through the parahippocampal gyrus with other temporal and extratemporal cortical association area. The name Cornu Ammonis (from Amun, an early Egyptian deity with a ram's head), dates back to the 18th century. In modern usage it excludes the dentate gyrus and is memorialized in terminology for sectors seen in transverse (coronal) sections of the hippocampus, with CA1 next to the subiculum and CA4 in the concavity (hilum) of the dentate gyrus (Kiernan, 2012; Tatum, 2012).

Given its central role within the limbic system, the medial temporal lobe establishes close connections with a diverse array of cortical structures, through both long and short association fibers as well as commissural fibers (Kiernan, 2012). Also, it exhibits important connections with subcortical structures as the thalamus and the hypothalamus, being the region which exhibits the highest number of functional links with the latter (Stephan Chabardès et al., 2002).

The neuroanatomical architecture of brain networks provides a skeleton of connected brain areas that facilitates signaling along specialized pathways to fulfill specific functions (Bressler et al., 2010). Within the structural connectivity of the temporal lobe, we can find the basis of its functional relevance, which is articulated through variations in its interactions with other areas (A. R. McIntosh, 2004). These interactions give rise to the functional networks, which can be altered in TLE, and which will be reviewed in detail in further sections.

1.3.2 A network disease

As posited earlier, the current conceptual definition of focal epileptic seizure implies that there is an underlying neuronal network, more or less distributed, but consistent among seizures (Berg et al., 2010): to reach success in TLE surgery this network has to be appropriately defined within the temporal lobe and disrupted with surgery; the structures encompassing the network and needed to be removed or disconnected are called the epileptogenic network, and can vary from patient to patient (Bartolomei et al., 2017).

The proposed network underlying TLE, so-called the "medial temporal/limbic network" is bilateral, cortical, and subcortical, and includes the hippocampi, the amygdalae, the entorhinal cortices, lateral temporal neocortices, and extratemporal components of the medial thalamus and the inferior frontal lobes. This set of structures is vital for sustaining seizure activity; besides, it is also a core network for cognitive and emotional processing (Spencer, 2002).

In recent years, approaching TLE as a brain network disease has been a framework hypothesis that improved the understanding of electrophysiological and imaging findings in patients with epilepsy. The brain is a complex network, evidenced by a plethora of neuroanatomic and neurophysiologic data. In this context, the idea that "focal" epilepsies are not in fact so focal, and involve networks of varying scales, has become progressively accepted in epileptology (Bartolomei et al., 2017). Clinical observations also support the concept of a large neural network in human epilepsy. Despite initial electrical pattern differences, clinical seizures exhibit a consistent pattern, highlighting the functioning of the epilepsy network. For instance, in the medial temporal/limbic network, seizures might start variably in specific locations (like the entorhinal cortex or hippocampus), but their clinical manifestation remains the same due to the network's collective involvement. Similarly, pa-

tients with medial temporal/limbic syndrome might experience seizures originating independently in both temporal lobes, yet the clinical appearance of these seizures is indistinguishable, emphasizing the network's overall role in seizure manifestation (Spencer, 2002).

1.3.3 Temporal lobe epilepsy: clinical picture and etiology

Patients suffering from symptoms of TLE can be traced back to ancient history, as it presents with highly identifiable symptoms, and it can be found well described in ancient texts (Patel et al., 2020). However it was not until more recently, in 1889, that an association between the clinical symptoms of TLE and hippocampal lesions was described (Jackson et al., 1889). The description of symptoms together with increasing knowledge of the structural and functional anatomy of the temporal lobe lead to first distinctions of mesial TLE, which is the predominant type, and neocortical TLE (Kennedy et al., 2012; Tatum, 2012). These distinctions correlate with the different structural and functional systems found within the temporal lobe, facts that together increase the heterogeneity of the disease.

Mesial TLE is a group of diseases that predominately involves dysregulation of hippocampal function (Tatum, 2012). As for its causes, HS is the most common disease encountered in epilepsy surgery series, and may be readily detected by brain magnetic resonance imaging (MRI) as mesial temporal sclerosis. The histopathologic hallmark of HS is segmental pyramidal cell loss, which can affect any sector of the CA, and is associated with a severe pattern of astrogliosis, conferring the Hippocampus a hardened consistency. Depending on the area and CA or subfields affected, four subtypes of HS have been described (Blümcke et al., 2013). Other causes of TLE that are less frequently seen include genetic anomalies, gliomas, angiomas, cavernomas, or traumatic or infectious; when these lesions co-exist with HS the term TLE with dual pathology is coined (Tatum, 2012).

Neocortical TLE is characterized by its etiologic heterogeneity and less typical electroclinical features than the more common mesial TLE (Gil-Nagel, 1997). The trademark of

neocortical TLE is a stronger involvement of temporal lateral cortex or adjacent structures, such as the insula, when compared with mesial TLE, but they share many common etiologies, as for instance HS (Kennedy et al., 2012).

In this sense, in many cases the clear cut difference between mesial TLE and neocortical TLE is not straightforward, as varying degrees of implication of the mesial and lateral structures can coexist leading to the distinction of temporal lobe and temporal lobe "plus" epilepsies (C. Barba et al., 2007; Carmen Barba et al., 2016). The most relevant implication is for patients undergoing surgery of the temporal lobe; brain network analysis can be a tool that gives insights not only on the extension of mesial or neocortical involvement, but also of extratemporal structures, overcoming the dichotomy of mesial or neocortical TLE (Bonilha, Martz, et al., 2012). These aspects of network measurements in temporal lobe surgery will be described in further sections.

1.3.4 Cognition in TLE

From an historical point of view, the first empirical studies of cognition in epilepsy began to appear in the early 1900s with a focus on the relationship between intelligence and clinical characteristics of the patients' epilepsy, and was accelerated by the development of epilepsy surgery programs (B. Bell et al., 2013). Later on, came the nowadays classic case of HM (and seven other patients), who following a bilateral temporal lobe resection, an extensive anterograde memory loss ensued with concomitant preservation of overall intellectual functioning and language ability (Scoville et al., 1957). This profile became regarded as the prototypical presentation of an amnesic syndrome produced by bilateral temporal lobe damage and opened the way for further studies. It also marks a significant milestone in distinguishing between TLE surgery and cognitive damage, a distinction that will be elaborated upon in subsequent sections of this thesis.

Next years saw several studies investigate into the cognitive profile of patients, combined with lesional studies, leading to more defined hypothesis on the structure-function relationship for TLE. The temporal lobe structures were known to play a pivotal role in memory processing, and its primary cognitive impairment was the disruption of declarative episodic memory, which involves knowledge linked to specific times and locations (B P Hermann et al., 1997). Semantic memory, which pertains to context-free world knowledge, was demonstrated to be also affected in TLE, but this impairment seemed to be more closely tied to dysfunction in the temporo-lateral neocortex rather than directly related to hippocampal pathology and dysfunction (Christoph Helmstaedter et al., 2012; Squire et al., 1991).

A significant body of data centered on episodic memory impairment and TLE. Focusing more on the evidence, it tended to exhibit material-specific patterns (verbal or nonverbal), depending on whether the left (language-dominant) or right hemisphere is affected (C. Helmstaedter et al., 1996). Studies also demonstrated significant correlations between test performance and several biomarkers in TLE. These correlations include structural markers such as hippocampal volume and hippocampal cell loss in distinct subfields, and also functional measures as event-related potentials, rhinal-hippocampal gamma-band EEG coupling and fMRI (B. Bell et al., 2013; Bonelli et al., 2010; Christoph Helmstaedter et al., 2012; Martin et al., 1998). In clinical practice, this implied that memory assessment in TLE served as a valuable indicator of temporo-mesial pathology and dysfunction, with even some potential lateralization depending on whether verbal or non-verbal impairment was found.

However, the evidence of more recent years has expanded this view, and has proven that patients with TLE can experience a range of cognitive issues, not limited to memory and language problems, but also affecting IQ, executive functions, language, and sensorimotor skills (B. Hermann et al., 2017). Interestingly, studies have found links between

structural and functional brain changes with cognitive performance beyond the temporal lobe. Global measures of structural integrity in the cortex, such as overall gyrification and brain volumes, showed correlations with global cognitive abilities (B. Bell et al., 2013). Additionally, subcortical structures like the thalamus and the basal ganglia, which are part of the interconnected brain network of TLE, could be also affected and relate to memory tasks (Stewart et al., 2009). On functional imaging, loss of connectivity in frontal lobe regions has been linked to executive dysfunction in TLE patients (Campo et al., 2013; Z. Zhang et al., 2009). On the other hand, the material specificity and lateralization model of mesial temporal lobe function has also been challenged (Saling, 2009). For instance, an study using fMRI demonstrated that while the association between verbal memory and the left temporal lobe seemed to be consistent in left TLE, the link between the right temporal lobe and figural or visuospatial memory appeared less consistent (Bonelli et al., 2010).

Amidst the more recent evidence suggesting a more heterogenous cognitive profile, there is some degree of differentiation. Cluster analysis has revealed three major cognitive phenotypes within the syndrome of TLE: generalized impairment, language & memory deficits and intact patients (Reyes et al., 2020). Further, the neural connections linked to each phenotype have revealed that individuals experiencing generalized impairment exhibit widespread brain abnormalities; those with memory and language deficits display more localized alterations within their temporal lobes and individuals with unimpaired cognition possess brains comparable to those of healthy controls. (Reyes et al., 2019; Rodríguez-Cruces et al., 2018).

More specifically, loss in functional connectivity among areas of the left hemisphere relevant to language and verbal memory (namely the hippocampus, inferior frontal gyrus, parahippocampus and lateral temporal region) have been linked to neuropsychological impairment of these domains (Roger et al., 2020). Also, atypical organization of functional

connectivity between the hippocampus and widespread cortical areas have been reported in patients with episodic memory impairment, suggesting that the restructuring of the hippocampus functional interplay with the broader brain network is central to the reorganization of memory networks, possibly leading to a less efficient functional framework (Q. Li et al., 2021).

Overall, cognitive impairment in TLE results from disruptions in the interconnected brain network rather than isolated brain damage, and the cumulative effect of these structural and functional abnormalities can lead to significant cognitive and behavioral challenges in these patients. Analyzing connectivity of key structures within the network can provide markers that relate with more specific language and memory domains, and help distinguish among the individual variability of patients with TLE.

1.3.5 Mood disorders in TLE

Traditionally, affective disorders in epilepsy were considered complications of the underlying seizure disorder. The "classic paradigm" explained the correlation between neurobehavioral comorbidity and epilepsy using lesion-related models. Indeed, according to this model, epilepsy comorbidity should be consequent to the epilepsy syndrome and its characteristics (etiology, onset, frequency, and treatment) (B. Hermann et al., 2017; Kanner, 2009).

More recent years saw the improvement in cognitive, neuroimaging, and clinical research that lead to a challenge to the classic paradigm (B. Hermann et al., 2017). Beginning with epidemiological studies that suggest a more complex relationship, indicating for instance that a history of major depression or anxiety disorders is associated with an increased risk of developing epilepsy (Kanner, 2009). Also, using a network related approach, recent evidence suggests that underlying mood disorders there is a dysfunction in a widespread network, which includes the medial prefrontal cortex (Murrough et al., 2016) and connected

limbic, striatal, thalamic, and basal forebrain structures, which play a crucial role in the development and persistence of affective morbidity (Kanner et al., 2012). This is supported by evidence from both structural and functional neuroimaging studies. Noteworthy, these same brain regions may be implicated in both seizure generation and the manifestation of affective symptoms in TLE (Stretton et al., 2015). In this sense, common structures and their interconnectivity are shared in TLE and mood disorders.

This evidence lead to the more new "bi-directional hypothesis," according to which behavioral disorders and psychiatric features may be considered not only a comorbidity but related to the same pathophysiology of epilepsy (B. Hermann et al., 2017; Vinti et al., 2021). This highlights the importance of addressing both the neurological and psychiatric aspects when managing patients with TLE (Stretton et al., 2015).

More specifically, several common structures are key to the neurobiological basis of TLE and mood disorders (Kaiser et al., 2015). The amygdala is determinant in the experience of fear and its autonomic and endocrine response (Sheline, 2003). The hippocampus, involved in reversal learning (Vilà-Balló et al., 2017, 2022) has been related to the pathogenesis of depression via changes in its volume and connectivity related to its plasticity, which have been found in chronic depression (Tartt et al., 2022). Also, extratemporal structures as the NAcc (Salgado et al., 2015) and the cingulate cortex (Rupprechter et al., 2020) are implicated in mood disorders, specifically in reward processing. Finally, reports show that alterations in connectivity among the mesial and extratemporal structures relate to depressive symptoms (Kemmotsu et al., 2014).

TLE is often accompanied by significant psychiatric comorbidity. The most common psychopathologies are mood disorders, such as depression and anxiety, which negatively impact the quality of life and increase the risk of suicide. Psychiatric morbidity in epilepsy

is high, with reported lifetime prevalence rates of up to 50%. This comorbidity influences quality of life, even independently of seizures (Kanner, 2009; Ramos-Perdigués et al., 2016).

The challenge also lies in understanding how surgical resection and therefore disconnection of this complex network of structures can lead to mood symptoms, as numerous other psychological and social factors also contribute to this phenomenon. In the next sections this topic will be further expanded.

1.4 Surgery for TLE

1.4.1 Primary outcome of surgery: seizure freedom

Surgery for TLE is among the safest and more effective procedures for refractory epilepsy. Noteworthy, a first clinical trial specifically for patients with TLE showed that performing a procedure called temporal lobectomy is superior to ASD management in drug resistant patients (Wiebe et al., 2001) (See Figure 7). Further, in 2012, a second trial also showed that temporal lobectomy was superior to ASD in controlling seizures for patients with TLE (Jerome Engel, 2012).

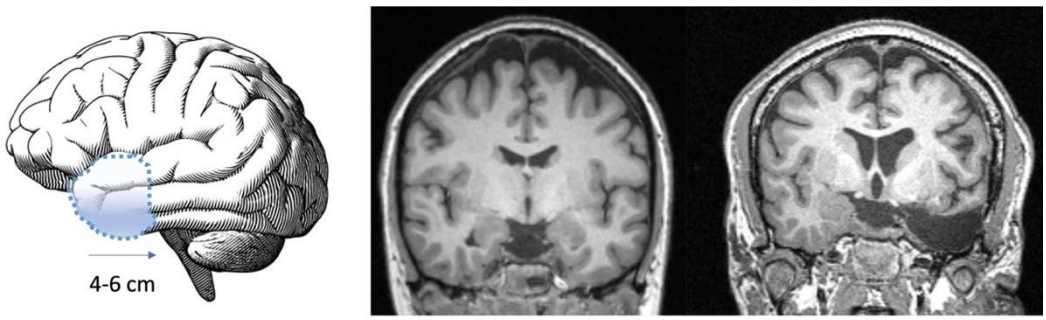


Figure 7 To the left, schematic representation of the region resected in a temporal lobectomy; according to Wiebe et al. (Wiebe et al., 2001), an *en bloc* resection of the temporal lobe, going back 4cm from the temporal pole for the dominant hemisphere, and 6cm from the temporal pole for the non-dominant hemisphere. To the right, two brain MRIs of the same patient, before and after undergoing a left temporal lobectomy.

Notwithstanding, up to 50% of patients who undergo a temporal lobectomy or anterior temporal resection surgery for epilepsy fail to remain long-term seizure free (De Tisi et al., 2011). Predicting which patients will benefit most from surgery has proven to be difficult, as TLE represents different entities (Carmen Barba et al., 2016; Bonilha, Martz, et al., 2012), and different patterns of relapse following surgery have been reported (Hennessy et al., 2000).

Initially for TLE patients, having a clear cut lesion, such as HS on MRI, was one of the factors considered to be related with good surgical outcome (Téllez-Zenteno et al., 2010). However, further studies have challenged the classic view that MRI lesions are associated

with greater chance of postoperative seizure freedom following surgery than normal MRI findings. One of these studies distinguished 'pure' TLE from so-called 'temporal plus epilepsy', characterized by an epileptogenic zone involving an extended area, including the whole temporal lobe as well as neighbored cortices including the operculo-insular region, the orbitofrontal cortex and the temporo-parieto-occipital junction (Carmen Barba et al., 2016). Noteworthy, this study provided evidence that this extended area or network was measurable with electrophysiological recordings and stretched beyond the limits of an MRI detectable lesion. Another reports using nuclear medicine imaging, also helpful in measuring the extension of the epileptogenic area beyond a detectable lesion on MRI revealed a relation with surgical outcome; having larger areas of alteration implied worse surgical outcome (Ryvlin et al., 2016). Finally, studies aimed at measuring network involvement with fMRI also revealed significant findings relating connectivity and surgical outcome in TLE surgery (He et al., 2017). Main findings of these studies revealed that larger network alterations, especially when implying areas outside the temporal lobe related to a poor outcome after surgery. This section will be expanded in the results section.

1.4.2 Other outcomes of surgery: cognition and mood

After the classic case of HM, along with seven other patients, who following bilateral temporal lobe resection couldn't form new memories or events after the surgery. However, other forms of memory were preserved in these patients despite the severe anterograde amnesia (Scoville et al., 1957). This landmark study provided an starting point in the understanding of the neural bases of memory and opened new lines of research in this field, taking the relation between cognitive assessment in TLE and outcome after surgical removal as a lesion model. Variability in memory outcomes after temporal lobe surgery was progressively reported, and several factors relating to these outcomes were concomitantly

put forward (B. Bell et al., 2013). Specifically, two primary factors contribute to this variability.

One major factor is the heterogeneity of memory tests used as well as the definition of cognitive domains: for instance, in the case of verbal memory, different memory tasks, such as list learning, paragraph recall, and word pair associations, have varying semantic demands and underlying neurobiological requirements, making them non-equivalent measures of verbal memory that could involve different brain regions (Saling, 2009). Following the case of verbal memory, even within similar tasks, the semantic relationships among stimuli words can influence sensitivity to dysfunction in the left temporal lobe, as the perirhinal cortex and the hippocampus contribute differently to these functions (Lillywhite et al., 2007). This has lead to international efforts in homogenization of test materials for patients with TLE who undergo surgery, (Vogt et al., 2017) and the challenge of a the modularity and material specificity of verbal and non-verbal memory systems (Saling, 2009).

Another source of outcome variability is the relationship between preoperative memory performance and the structural and functional status of the to-be resected hippocampus. Initial studies found that the structural integrity of the to-be-resected hippocampus could predict the risk of postoperative memory changes, with greater risk in patients with a more functionally intact hippocampus and lesser risk in those with more extensive hippocampal cell loss (B P Hermann et al., 1992). This finding contradicted the conventional belief that the risk of postoperative memory decline was associated with the functional integrity of the contralateral hippocampus. In 1995, Chelune (Chelune, 1995) proposed two contrasting models regarding memory outcomes in patients undergoing hippocampal surgery. The "hippocampal adequacy model" suggested that the functional state of the surgical hemisphere and hippocampus prior to surgery determined memory outcome. Patients with a

more intact hippocampus were at a greater risk of memory decline because relatively functional tissue was removed. On the other hand, the "functional reserve model" emphasized the importance of the contralateral non-surgical hippocampus. If the contralateral hippocampus remained intact and capable of supporting memory functions, it could compensate for the loss of the ipsilateral (surgical) hippocampus. Numerous findings have supported the hippocampal adequacy model, mainly based on structural MRI by measuring the volume of the hippocampus (S. A. Baxendale et al., 2000).

As mentioned earlier, in recent years the investigation of cognitive networks in which the hippocampus assumes a pivotal role, measured with functional MRI and assessments of network metrics, has yielded enhanced outcomes in predicting cognitive disabilities (G. E. Doucet et al., 2015; Mccormick et al., 2013; Sidhu et al., 2015). In this sense, the integration of hippocampal connectivity within extended brain networks crucially plays a role in sustaining cognition (Rodriguez-Cruces et al., 2022). This section will be expanded in the results section.

Furthermore, the affective domain can also be affected after temporal lobe surgery as mood disorders. Although there is less reports in the literature about mood outcome after surgery (Cleary et al., 2013), several studies have investigated whether mood improves or worsens after surgery and a complex interplay of different factors has been identified as playing a role in mood outcome after surgery (Cleary et al., 2013). Over fifty percent of individuals with treatment-resistant conditions exhibit psychiatric disorders (Ramos-Perdigués et al., 2018). These conditions significantly diminish their quality of life, an impact that remains substantial even when considered independently of seizures. Furthermore, psychiatric disturbances often arise following surgery, either as new-onset symptoms or as a worsening of pre-existing ones (Ramos-Perdigués et al., 2016). Surprisingly, despite these well-documented occurrences, there are currently no established protocols for conducting

psychiatric assessments before or after surgical interventions (Cleary et al., 2013). Recognized risk factors for postoperative depression encompass a history of preoperative depression, a family history of psychiatric disorders, advanced age, resections involving mesial temporal structures, the presence of multilobar and bilateral epileptiform discharges, a history of secondarily generalized tonic-clonic seizures, longer disease duration, maladaptive family dynamics, financial instability, and unemployment (Doherty et al., 2021). This section will be expanded in results section.

Finally, as mentioned earlier, patients with TLE may also suffer from cognitive problems and mood decline already before surgery, because of the enduring seizures but also depending on the etiology of the epilepsy. Therefore, it is a complex situation whether a patient with TLE will benefit completely or just partially of a temporal lobectomy.

A converging perspective is to assess the validity of network connectivity biomarkers that could help in the prediction of cognitive and mood outcomes of surgery. This section will be expanded in the results section.

1.4.3 Outcome of surgery: prediction at individual level?

The quest to understand the potential advantages and challenges associated to epilepsy surgery within the heterogenous nature of TLE has been intrinsically related with the evolving history of conceptual definitions and frameworks for epilepsy. Over the years, several distinctions have been introduced to better classify patients, such as new classification schemes and subtypes of TLE, in an attempt to increase precision in treatment and prognosis (C. Barba et al., 2007; J. Engel, 1992; H.-G. Wieser et al., 2004).

However, accurate predictions concerning surgical risks associated to seizure relapse and cognitive/psychiatric impairment for individual patients remains to pose significant

challenges. One possible explanation for these difficulties lies in the prevalent use of uniform, one-size-fits-all analytical framework at the group level (Lo et al., 2015). While the identification of consistent and reliable average distinctions is undoubtedly valuable, this approach predominantly emphasizes shared trends and overlooks the nuanced interindividual variations across the entire disease spectrum (Lee et al., 2022).

In contrast, there is a growing acknowledgement of the importance of analyzing diversity among patients, often referred to as inter-patient heterogeneity. This shift towards recognizing inter-patient heterogeneity as a crucial aspect of healthcare is increasingly seen as a significant step towards adopting a more person-centered approach to medical care (Sisodiya, 2021). By delving into the unique characteristics and needs of individual patients, healthcare professionals can better tailor their interventions and treatments, potentially leading to more precise predictions of surgical outcomes, cognitive functioning and even drug resistance on a case-by-case basis. This approach holds the promise of enhancing the overall quality and effectiveness of healthcare delivery.

While the majority of studies in the realm of TLE have primarily focused on investigating the differences that exist between patients and control groups as a whole (Lee et al., 2022), there is a growing awareness that these case-control study designs might not effectively capture the biologically and clinically significant distinctions among individual patients (Lo et al., 2015). In light of this perspective, the pursuit of data-driven discovery at the individual level presents innovative opportunities and pathways for patients with TLE (Lee et al., 2022). The network approach, through brain network analysis has revealed several alterations that enlighten the understanding of surgery's potential outcome (Bernhardt et al., 2019). The assessment of individualized network biomarkers provides a promising new avenue in the clinical care of epilepsy, namely the evaluation of the clinical trajectory based on the person's unique neural architecture.

1.5 Brain network analysis

Evidence from the 19th century has already substantiated the notion that the neural components within the brain form an intricately complex network of significant magnitude. Since the advent of the 20th century, there has been a widespread recognition that this anatomical framework facilitates the dynamic manifestation of coherent physiological activity, encompassing numerous distinct brain regions, which collectively constitute a functional network. It is widely postulated that such networks serve as the physiological underpinnings for the processing of information and the formation of mental representations (Bullmore et al., 2009, 2012). In the context of TLE, the pathological epileptogenic activity disrupts the normal brain network dynamics, and may alter the connectivity patterns and therefore affecting its overall function (Cataldi et al., 2013; D. W. Gross, 2011).

There are several methodological approaches available to assess brain network connectivity at different brain scale. At a macroscopical level, through neuroimaging, specifically with brain MRI, we can measure structural and functional connectivity with DTI and fMRI, respectively (Bressler et al., 2010).

1.5.1 Structural MRI

In neuroscience research, structural MRI is typically used to obtain volume measures of the brain. In this regard, the first evidence from a pathological network of structures involved in TLE emerged from volumetric studies (Simon S Keller et al., 2009). Using quantitative MRI tools that initially focused on manually delineated volumes to assess brain size, researchers first examined structures associated with the hippocampus. They found atrophy not only in hippocampal regions but also in neocortical temporal lobes, entorhinal cortex, fornix, parahippocampal gyrus, and amygdala (Simon Sean Keller et al., 2008). Progressively, automated methods such as voxel base morphometry improved and analysis at

whole-brain level were implemented. With these approaches, grey matter loss of extratemporal regions as the parietal lobe and in subcortical structures as the basal ganglia, and the thalamus was detected across studies (Stewart et al., 2009). Thus, further evidence demonstrated that the pattern of brain atrophy in TLE was extended beyond the epileptogenic zone, predominantly ipsilateral to the seizure focus in the temporal lobe (Focke et al., 2008). However, a more bilateral distribution of extratemporal lobe and subcortical abnormalities was also found (Bonilha & Keller, 2015).

These findings contributed a shift in research focus within TLE towards brain network analysis. For this purpose, structural MRI covariance has been used, applying graph theory to segmented brain regions. Researchers observed abnormalities in network patterns, cortical atrophy, and changes in network hubs among TLE patients compared to controls (Bonilha & Keller, 2015).

Regarding the relationship of structural MRI and TLE surgery, initially, quantitative MRI techniques were used to study the hippocampus, particularly its volume, morphology, and structural changes, in relation to TLE (Arruda et al., 1996; Simon S. Keller et al., 2015). Some studies suggested that a larger hippocampal volume on the side of resection may lead to poorer postoperative seizure outcomes, while others propose that significant volume reduction in the hippocampus was associated with better outcomes (Bonilha & Keller, 2015). Then, the application of whole-brain methods provided evidence that bilateral hippocampal alterations, along with alterations in regions outside the hippocampus, such as the entorhinal cortex, temporopolar and insular cortices, and thalamic structures, might play a role in persistent postoperative seizures (Bonilha & Keller, 2015).

1.5.2 Resting-state functional MRI

fMRI is a noninvasive neuroimaging technique that captures Blood-oxygen-level-dependent (BOLD) signals in the brain in vivo. The BOLD signal is a measure of metabolic brain activity based on the difference between oxyhemoglobin and de-oxyhemoglobin levels arising from changes in local blood flow, relating to neuronal activation. fMRI can be performed during active task, in order to evaluate the neuronal correlates associated to this task, or during periods of rest, leading to the coining of the term rs-fMRI (Bressler et al., 2010; Tousseyn et al., 2015).

In rs-fMRI the network dynamics of the whole-brain can be analyzed, and a plethora of statistical methods can be used to study the data. The concept of RSN originated from the seminal work of Biswal (Biswal et al., 1995) who were investigating the source of noise in rs-fMRI signals during a simple motor task in healthy participants. Using an experimental paradigm that alternated finger tapping and rest periods, they identified low frequency fluctuations in the motor cortex unrelated to heartbeat or respiration. These fluctuations occurred at rest and showed a high degree of correlation, with similar fluctuations in contralateral motor cortex. Further work revealed that they were caused by spontaneous changes in the BOLD signal, representing an index of oxygen and blood flow dependent on neural and glial activity (van den Heuvel et al., 2010). From then on studies has showed that multiple regions show coherent BOLD fluctuations and therefore are believed to be functionally connected and form discrete resting state networks (Damoiseaux et al., 2006). Although resting state networks have been identified due to their activity at rest, they are also relevant for many other aspects of normal brain functioning and there is correspondence with task-related networks (Smith et al., 2009).

Evidence suggests that TLE could modify chronically the activity of brain networks that control basic functions such as attention, emotion, and cognition (Rodriguez-Cruces et al., 2022). The involvement of large extratemporal networks could explain, for instance,

the appearance of the several cognitive or psychiatric complications of TLE. (Cataldi et al., 2013). Also, the epileptogenic network might have its own signature in rs-fMRI, and a variety of methods to identify and localize it have been proposed and could help in predicting seizure outcome after surgery (Jackson et al., 2017; Maneshi et al., 2014)

1.5.3 Diffusion tensor MRI

DTI is a class of noninvasive MRI techniques that trace fiber bundles (white matter tracts) in the human brain in vivo based on properties of water molecule diffusion in the local tissue microstructure (Beaulieu, 2002). The basic assumption when interpreting diffusion-related measurements is that water diffusion in white matter experiences restriction perpendicular to the direction of white matter tracts, due to barriers to water diffusion such as axon membranes and myelin, while parallel diffusion is relatively unimpeded (Le Bihan et al., 2006).

Therefore, decreased asymmetrical water diffusion and reduced membranes or myelin, measured as FA and MD respectively, are interpreted as reflecting reduced integrity of the white matter tract. Along with providing a noninvasive measure of white matter integrity, DTI can also provide a means to extract the three-dimensional (3D) location of specific white matter tracts (DTI tractography), which provides the opportunity to study specific white matter tracts that are difficult to precisely localize with other imaging techniques. (D. W. Gross, 2011). Also, DTI provides high microstructural contrast, being able to detect alterations in gray matter and in the hippocampus proper (Salo et al., 2017)

As with rs-fMRI, DTI allows us the study of brain connectivity at a whole brain basis. Regarding TLE, DTI reported findings strongly suggest that in TLE structural abnormalities are not restricted to the ipsilateral hippocampus and temporal lobe white matter, but rather involve an extensive bilateral network of structures (D. W. Gross, 2011; Miró et al.,

2015). Patients may present with overlapping anomalies in MD and FA, particularly in ipsilateral temporo-limbic regions but also relating to distant regions (i.e. anterior and posterior midline regions, lateral temporo-parietal cortices) (M. Liu et al., 2016), as well as white matter tracts, and may relate to disease duration (Miró et al., 2015) and cognitive impairment (B. Bell et al., 2013)

1.5.4 Evidence from other modalities

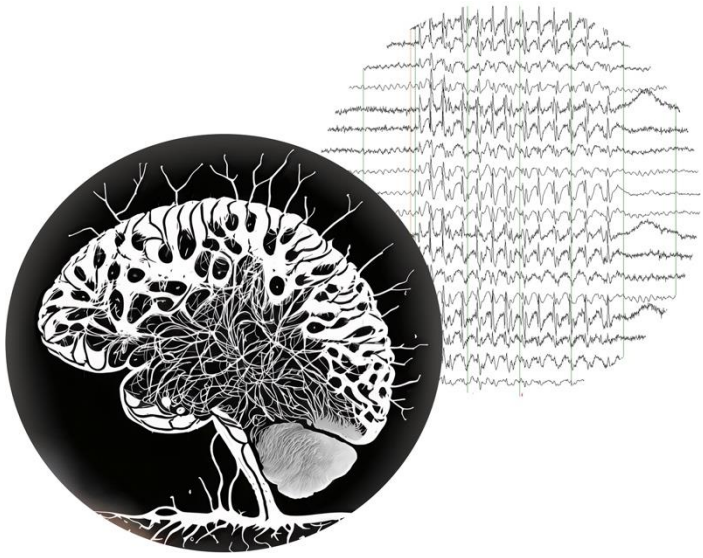
As mentioned previously, the observation of intracranial EEG in epilepsy patients strongly supports the network hypothesis for seizures. The entire interconnected network contributes to seizure expression, and seizures may be triggered from different parts within the network (Bartolomei et al., 2017). The concept of a single "seizure onset" area is misleading, as onset could occur anywhere in the network, even varying between seizures in the same patient. This variability in location may lead to distinct seizure onset patterns when EEG is recorded from only one network segment (Spencer, 2002).

Neuronal dynamics at the microscale, via recordings of neural units, have been a contributing force to the concepts of neural networks and patient heterogeneity in epilepsy. Within epileptic tissue, there is a high presence of functional neuronal clusters, and these clusters exhibit distinct patterns of spatial localization (Feldt Muldoon et al., 2013a). This implies that network events are not merely the result of cells becoming overly synchronized, but instead represent a phenomenon characterized by interactions among changing cell assemblies dispersed throughout space (C. J. Keller et al., 2010). An implication of this is that various combinations of active neuronal clusters generate pathological interictal events, which might seem to recur or repeat themselves only when observed from a macroscale perspective (e.g., through EEG or local field potential recordings), and can affect spatially separated areas (Szabo et al., 2015). The intricate behavior of this network at the microscale, is mirrored at the macroscale. Through intracranial EEG, seizures manifest as a result of

the intricate interplay involving both synchronization and desynchronization of assemblies of neurons that are spatially distant. But interconnected along structural and functional pathways and may involve both lesional and non-lesional tissue (Bartolomei et al., 2017; Chauvel et al., 2014; Spencer, 2002).

Finally, functional nuclear imaging, particularly PET scans, have significantly contributed in advancing the understanding of epileptic networks in human epilepsy (Yakushev et al., 2017). While seizure propagation is distinct from the generating network, nuclear imaging plays a crucial role in understanding epileptic networks by circumventing the complexities associated with propagation (Van Paesschen et al., 2007). In the context of the medial temporal/limbic network syndrome, PET reveals variable and widespread interictal hypometabolism involving multiple structures, such as the ipsilateral temporal neocortex, hippocampus, thalamus, and frontal lobe (Duncan et al., 2016). This interictal hypometabolism appears consistent and defining of the medial temporal/limbic network. Successful surgery for this syndrome leads to improvements in hypometabolism, suggesting a direct link between the disruption of the structural network and seizure cessation (Spencer, 2002).

RESEARCH AIMS



2 RESEARCH AIMS

The aim of the current doctoral thesis is to evaluate, at an individual level, which patients with TLE can benefit most of epilepsy surgery across different domains: seizure freedom, cognitive decline and mood disorders. To achieve this, we will identify specific biomarkers within the epileptic brain from a network perspective in patients with focal, drug resistant epilepsy originating in the temporal lobe. Then, we will use this information to make a personalized prediction of surgery risks in the above mentioned domains. This evaluation involves the integration of different MRI modalities with neurological and neuropsychological assessments.

This doctoral thesis is structured around four studies aligned with two specific objectives:

1. **Identify specific brain network biomarkers**: Utilize advanced neuroimaging modalities and techniques to quantify the affected temporal lobe function pre-surgically by examining its interactions with different brain networks in TLE patients.
2. **Explore prognostic use of biomarkers:** Investigate the potential prognostic value of these biomarkers in assessing the risks of TLE surgery outcomes, in terms of seizure relapse and cognitive and mood outcomes, in a single-subject level.

By addressing these objectives in specific research questions, the thesis aims to contribute valuable insights into the personalized evaluation of epilepsy surgery outcomes, considering the intricate interplay between the affected hippocampus and seizure control, cognitive function, and mood regulation in patients with TLE.

2.1 Research questions and hypothesis

2.1.1 Do patients with different connectivity patterns before surgery have different surgical outcomes in terms of seizure freedom?

The first two studies of the thesis aimed to answer this question. Along the framework of measuring different parts of the epileptogenic network underlying seizures, we selected new structural and functional biomarkers that could be related with seizure outcome. In Study 1, microstructural related measures of the hippocampal subfields contralateral to resection side were analyzed. The hypothesis of the study was that patients with less integrity in specific subfields would experience relapse after surgery. In Study 2, we identified a new functional biomarker, the DC, that accounted for the global connectivity at voxel-level. The value of this biomarker was explored with the hypothesis that a greater network abnormality would result in a poorer surgical outcome.

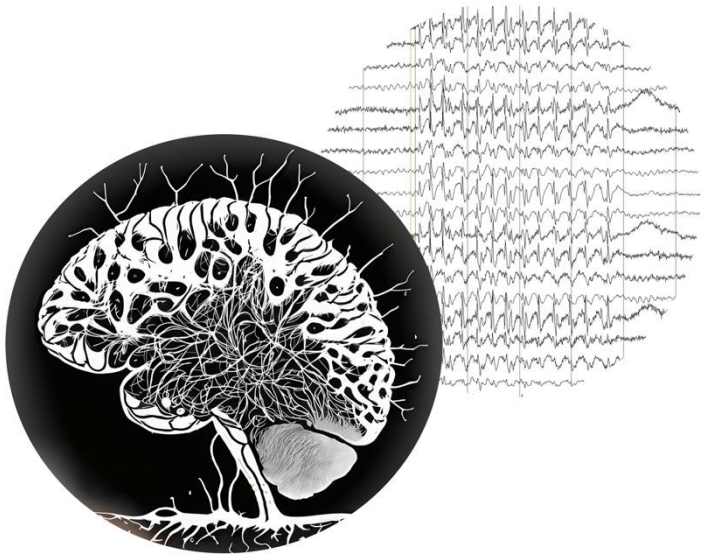
2.1.2 Does functional connectivity prior to surgery relate to cognitive outcome?

To address this question, in Study 3 we compared the functional connectivity along the longitudinal axis of both hippocampi with several cognitive-related resting state networks between patients who exhibit cognitive decline after surgery and those who did not. The hypothesis was that different connectivity patterns could be detected among patients with cognitive decline even before surgery.

2.1.3 Could functional connectivity before surgery influence mood decline?

In Study 4, we investigated the connectivity of key structures potentially affected in the network underlying TLE and mood disorders: the hippocampus and the NAcc. The hypothesis was that this connectivity could differ prior to surgery, particularly in patients with no previous history of psychiatric disorders and mood decline after the procedure. The observation of mood decline in these patients raised the possibility of mood symptoms emerging when the network is disrupted due to surgery.

METHODS



3 METHODS

This thesis investigates the utilization of rs-MRI and DTI to extract individualized biomarkers and assess their predictive value in determining the outcomes of epilepsy surgery. Comprising four distinct studies, each focuses on elucidating the role of a specific biomarker in various surgical outcomes for TLE. In the subsequent results section, an in-depth exploration of the methodology employed in each study will be provided, outlining the unique experimental designs and specifications.

3.1 General Methodology

Across the four studies, biomarkers were derived from pre-surgical DTI or rs-MRI sequences. Patients were categorized based on the specific outcome under evaluation, such as seizure freedom, cognitive decline, or mood changes. Subsequently, the significance of each biomarker was evaluated at the individual level to ascertain its predictive value prior to surgery.

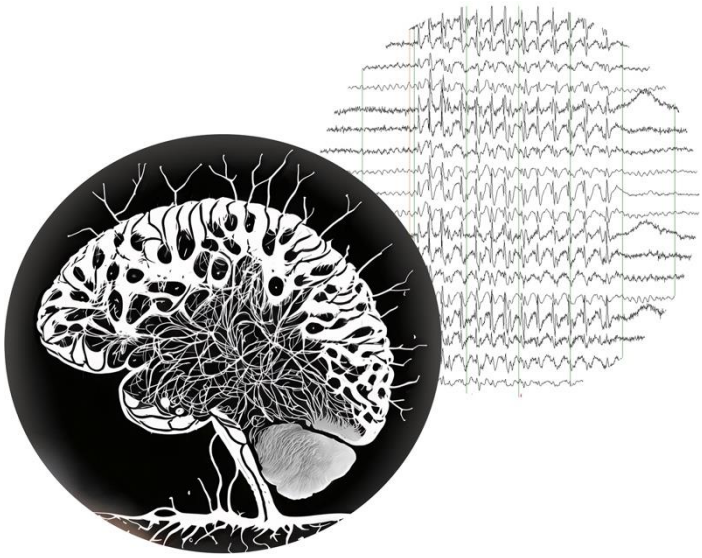
Significantly, the entire patient cohort was longitudinally tracked within a single center over an extended duration after surgery. This aspect holds particular importance when assessing post-surgical seizure freedom, as some patients may experience relapses even years after surgery, albeit with diminishing likelihood over time (De Tisi et al., 2011). For cognitive and mood outcomes, precise assessments were conducted at designated intervals to evaluate changes, with detailed descriptions provided in the experimental design of each study in the subsequent section.

In each study, various software packages were utilized for MRI analysis. Each subsection will comprehensively describe these software tools and outline their specific utility within the experiments. To ensure reproducibility, meticulous details regarding the software packages and the MRI processing and analysis procedures will be provided.

A primary objective of this thesis is to address the challenge of patient heterogeneity by introducing novel biomarkers that hold validity at the individual level. To this end, two statistical methods used in the thesis must be highlighted, the Crawford T-test and the ROC analysis. The Crawford T-test is a statistical test specifically designed to compare the performance of an individual or a small group of individuals with a control or normative group. It is particularly useful when dealing with cases where the data distribution violates assumptions of parametric tests, such as normality or homogeneity of variance (J. Crawford et al., 1998). Besides, the ROC analysis was employed in most studies to establish cut-off points, facilitating the integration of DTI and rs-MRI sequences with these new biomarkers into the pre-surgical assessment of TLE surgery candidates.

By leveraging these advanced imaging techniques and individualized biomarkers, the methodology employed strives for replicability, ensuring a robust framework to enhance the precision and efficacy of individual patient selection for epilepsy surgery. Ultimately, this approach aims to optimize individual treatment outcomes and enhance patient care.

RESULTS



4 RESULTS

4.1 STUDY 1: Hippocampal microstructural architecture and surgical outcome

Our aim was to study the microstructural architecture of the contralateral hippocampus to the affected side in patients with TLE-HS and its relation with surgical outcome.

For this purpose, we included 33 consecutive patients evaluated in our epilepsy surgery program during a five-year period. They underwent a presurgical MRI with volumetric T1 and diffusion weighted sequences. 22 patients with TLE-HS (13 women, 12 right TLE-HS) were finally selected. Median follow-up after surgery was 6.25 years (4.5-8.83 years). We segmented the hippocampal subfields of the contralateral hippocampus using FreeSurfer and calculated the FA and the MD of each subfield. We also scanned 18 healthy age-matched controls.

After surgery, 50% of the patients (n=11) remained SF following surgery. Comparing non-SF to SF patients, the MD showed increased values of the CA1 ($p=0.035$), the molecular layer ($p=0.010$) and the dentate gyrus ($p=0.041$) in the healthy hippocampus. Using a cut-off point for a survival analysis, we found that patients with lower values of MD of the molecular layer and the CA1 remained SF during long-term post-operative follow-up ($p<0.0001$).

As per our results, we concluded that the contralateral hippocampal internal microstructure may have implications in post-surgery seizure freedom in patients with TLE-HS. This study corresponds to: Sala-Padro, J., Miró, J., Rodriguez-Fornells, A., Quintana, M., Vidal, N., Plans, G., Santurino, M., Falip, M., & Camara, E. (2020). Hippocampal microstructural architecture and surgical outcome. Seizure, 76, 84–88. doi: 10.1016/j.seizure.2020.01.00.

4.1.1 Background

Patients with TLE who undergo epilepsy surgery aim at seizure freedom. Current state of evidence suggests that biomarkers extracted from both DTI and rs-fMRI could help in stratifying the odds of seizure freedom after surgery.

Previous neuroimaging studies have mainly placed the focus on characterizing the affected hippocampus, since surgical removal of the mesial temporal structures has proven to be effective; however, approximately 40% of patients relapse in the long-term (Mathon et al., 2017).

In some cases, measuring abnormalities affecting the contralateral hippocampus to the affected side arise as a possible marker of risk for persistent postoperative seizures, suggesting a bilateral asymmetric disease in some patients (So et al., 1989). Changes in the contralateral mesial structures of patients with unilateral TLE-HS using structural and diffusion imaging have been noted (D. W. Gross, 2011; Lin et al., 2005). It has been proposed that this findings are related to seizure propagation patterns (Miró et al., 2015), the progressive nature of TLE-HS and may relate to seizure relapse after surgery (Bernhardt et al., 2013). Furthermore, studies on the extent of TLE-HS have shown a relationship between bilateral mesial abnormalities in white matter tracts and seizure relapse, proposing that the extent of the TLE-HS network disease influences the surgery outcome (Simon S. Keller et al., 2017). In this sense, measuring the bilateral abnormalities in patients with TLE-HS could help stablishing a prognosis before surgery (Bernhardt et al., 2013; Simon S. Keller et al., 2017).

However, previous post-mortem pathological brain tissue analysis reported bilateral reorganization of the dentate gyrus in patients with TLE-HS, but authors postulated it to be a result of persistent seizures rather than contributing to seizure generation (Thom et al., 2009). Currently, there is a lack of studies focused on the contralateral hippocampal subfield abnormalities of TLE-HS patients.

As mentioned earlier, DTI in animal models have demonstrated how FA and MD relate to hippocampal damage as well as disease evolution and severity. In a TLE mouse model, DTI proved to be sensitive to histological changes related to epileptogenesis and its progression, mainly in the dentate gyrus and the CA1(Janz et al., 2017; Sierra et al., 2015) reporting increases in MD of these subfields. Also, different network reorganization has been found underlying different types of epilepsy (Sierra et al., 2015). Using diffusion imaging in human epileptic patients, the assessment of hippocampal subfield diffusion has shown a strong relation with neuronal loss in the hippocampus, MD being the most prominent marker of neuronal density (Goubran et al., 2016).

Based on this evidence, we aimed to evaluate the relation between the hippocampal subfields microstructure of the contralateral hippocampus in patients with TLE-HS and long-term seizure outcome after surgery using DTI, by extracting the FA and MD values of the subfields and comparing them among patients who underwent surgery.

4.1.2 Experimental design

The study recruited 33 consecutive patients (20 women) with refractory unilateral TLE-HS, evaluated in our surgery program from June 2009 to March 2014, who were considered candidates for surgery (anterior temporal lobe resection). Diagnosis was established according to clinical, EEG and MRI data (H.-G. Wieser et al., 2004). All patients underwent neurological and neuropsychological examination, video-EEG monitoring with seizure recording and structural brain MRI with the study protocol prior to surgery. Of these initial 33 patients, 31 patients underwent surgery, and 24 had proven TLE-HS on tissue sample. Of these, two patients were discarded due to erroneous acquisition of diffusion data. All patients were operated on by the same surgeon, and anatomopathological analysis was done by the same pathologist.

Finally, 22 patients (13 women) were included in the study; median duration of epilepsy was 33 years (6-59 years), and 12 had right TLE-HS. Median time of follow-up after surgery was 6.25 years (4.5-8.83 years), with all patients regularly undergoing prospective follow-up in our epilepsy clinic every three to six months. 50% of the patients (n=11) remained as completely SF at the end of follow-up, 8 of them being (72%) without antiepileptic drugs. We also included 18 healthy individuals matched for handedness, age, gender and years of education. This study was approved by the Ethical Committee of Hospital of Bellvitge.

All participants underwent a pre-operative whole-brain structural MRI scan using a 3.0 Tesla Siemens Trio MRI. A 32-channel phased-array head coil system was used to acquire high-resolution T1-weighted images (slice thickness=1mm; no gap; number of slices=240; TR=2300ms, TE=3ms, matrix=256x256; FOV=244mm; voxel size 1x1x1mm). A DTI sequence was carried out using diffusion tensor spin-echo planar imaging with coverage of the whole head (voxel size of 2.5x2.5x2.5mm, matrix of 96x96, 55 slices with 2.5 mm-thick

and no gap, TE=98ms, TR=9600ms, EPI factor=96, field of view=240mm, band-width=1022Hz, echo-spacing=1.08ms, b-value=1000s/mm2). For the DTI data, one single run of 64 diffusion-weighted directions with one non-diffusion-weighted volume was acquired.

Total hippocampal volumes were segmented from a fully automated pipeline for hippocampal subfields including the automated cortical parcellation and subcortical recon-all tools implemented in FreeSurfer 6.0 (http://surfer.nmr.mgh.harvard.edu/). The technical details have been described previously (Iglesias et al., 2015). In summary, the hippocampal subfields were segmented using a Bayesian inference approach and a novel atlas algorithm of the hippocampal formations. The contralateral hippocampi were segmented into thirteen subfields including CA1, CA2/3, CA4, granule cell layer of the dentate gyrus, fimbria, subiculum, presubiculum, parasubiculum, molecular layer, hippocampus-amygdala-transition-area (HATA), hippocampal tail, whole hippocampus and hippocampal fissure. To adjust for differences in head size, all volumes were normalized to ICV by dividing by the ICV calculated using FreeSurfer. Finally, all generated hippocampal segmentations were visually inspected to ensure there were no technical failures. Figure 11 illustrates the subfield segmentation of one patient.

Diffusion-weighted images were automatically processed using FreeSurfer 6.0 software (http://surfer.nmr.mgh.harvard.edu/). More specifically, head motion and eddy-current correction were first performed using the FMRIB's Diffusion Toolbox (FDT) in FMRIB's Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl/fdt) and the gradient matrix was rotated accordingly (Leemans et al., 2009). Then, FreeSurfer's boundary-based registration method was used for intra-subject alignment between the diffusion-weighted and anatomical images, and to an MNI152 template (Greve et al., 2009).

The diffusion tensor was then reconstructed using a standard least square tensor estimation algorithm for each voxel and its corresponding eigenvalues and eigenvectors were extracted to calculate FA and MD. Subsequently, the individual hippocampal subfields of each participant were registered to the individual FA maps using the transform provided by FreeSurfer. Then, the mean FA and MD values for all hippocampal regions of each subject were extracted using Freesurfer tools. Lastly, we obtained the volume, FA, and MD values for the contralateral subfields.

Statistical analyses were performed in SPSS v.22 (SPSS Inc, Chicago, USA). Independent two-tailed Student's t-test were used to describe clinical and sociodemographic differences between groups. The hippocampus volume and microstructure of each subfield were checked for normality using Q-Q plots. Then, ROC curves were configured in order to calculate cut-off points for variables showing significant differences on the t-test, with best sensitivity and specificity to predict recurrence. A model using Cox-regression was performed in order to analyze which variables were independent predictors of recurrence. Finally, recurrence during follow-up was analyzed with the Kaplan-Meier product limit survival method using the log-rank test to determinate statistical significance between groups. A p-value <0.05 was considered statistically significant.

4.1.3 Results

Demographic and clinical characteristics for the 11 SF patients, 11 non-SF patients and the control group are shown in the table below (see Figure 8). When comparing SF and non-SF groups, no differences were found in age of onset (p=0.6), history of generalized tonic-clonic seizures (p=0.692), duration of epilepsy (p=0.290) or history of febrile seizures (p=0.615).

	Seizure-free (n=11)	Non-seizure free (n=11)	Controls (n=18)	*p*
Age. Mean y-o (SD)	46.42 (10.46)	42.67 (12.86)	49.50 (11.92)	0.310
Women	7 (63.6%)	6 (54.5%)	11 (61%)	0.665
Left HS	5 (45.5%)	5 (45.5%)		0.168
Age of onset of seizures. Mean y-o (SD)	11.25 (9.52)	13.67 (12.5)		0.290
Duration of epilepsy Mean years (SD)	35.16 (12.71)	29 (15.06)		0.6
Febrile seizures	2 (18.1%)	3 (27.2%)		0.615
Secondary generalisation	9 (81.8%)	10 (90.9%)		0.692
ILAE outcome	1 – 11 (100%)	2 – 2 (18.2%) 3 – 8 (72.7%) 4 – 1 (9.1%)		
WMS – immediate verbal	24.58 (8.35)	29.09 (8.14)		0.205
WMS – delayed verbal	14.42 (6.65)	14.82 (6.03)		0.882
WMS – immediate visual	68.67 (11.9)	78.45 (20.73)		0.175
WMS – delayed visual	47.83 (18.44)	49.91 (21.65)		0.913

Figure 8. Patient and control baseline characteristics.

y-o = years old, SD = standard deviation, HS = Hippocampal sclerosis, WMS = Wechsler memory scale. For the WMS, direct punctuations are shown in mean values and standard deviation in brackets.

Regarding hippocampal subfield segmentation of patients compared to controls, patients had significant atrophy of the whole hippocampus and all the subfields on the affected side, along with the contralateral pre-subiculum. Significant changes of FA and MD in a variety of the healthy subfields was also found when comparing patients to controls (see Figure 9).

Subfield	Volume		FA	MD
	Healthy side	Affected side	Healthy side	Healthy side
Whole hippocampus	0.11	-8.50***		
Hippocampal tail	-0.80	-5.96***	-2.30	0.77
Subiculum	-1.22	-8.24***	2.38*	-2.52*
CA1	1.29	-8.07***	1.81	-1.55
Presubiculum	-2.06*	-6.54***	-3.00**	0.22
Parasubiculum	-0.47	-2.36*	-3.00**	1.08
Molecular layer	0.23	-8.46***	-3.12**	1.36
Dentate gyrus	1.18	-7.80***	-1.82	0.56
CA3	1.69	-6.83***	0.086	-0.768
CA4	1.00	-8.05***	-2.86*	-1.37
Fimbria	-0.263	-1.90	-1.40	1.96
HATA	1.74	-2.25*	-2.44*	-0.327

Figure 9. T values for comparisons among the subfields of patients and controls.

***p<0.05, **p<0.01, ***p<0.001**

We then compared diffusion parameters for the two groups of patients, SF and non-SF. The comparison showed statistically significant increases of MD in the non-SF patients on the CA1 (p=0.035), the molecular layer (p=0.010) and the dentate gyrus (p=0.041) of the contralateral hippocampus (Figure 10).

Subfield	FA	MD
	Healthy side	Healthy side
Hippocampal tail	1.03	-0.53
Subiculum	-0.325	-1.36
CA1	-1.00	-2.26**
Presubiculum	-0.39	-0.91
Parasubiculum	-0.19	-1.76
Molecular layer	-0.83	-2.83***
Dentate gyrus	0.81	-2.18**
CA3	1.07	-0.78
CA4	-0.33	-1.22
Fimbria	-0.91	0.11
HATA	-1.14	-0.22

Figure 10. T values for comparisons among the subfields of seizure free vs. non-seizure free patients. *p<0.05, **p=0.01

We finally examined at which cut-off point the different diffusion values better predicted seizure freedom. Using a Cox-Regression model, both the MD of the CA1 (p=0.026, B=11.39, SE=5.10) and the MD of the molecular layer (p=0.008, B=7.11, SE=2.7) were found to be independent predictors of seizure recurrence. Using these cut-off points for a survival analysis, we found that patients with lower values of MD of the contralateral molecular layer (MD<0.001) and the CA1 (MD<0.0008) remained seizure free during long-term post-operative follow-up, showing a significant difference from non-SF patients (See Figure 11).

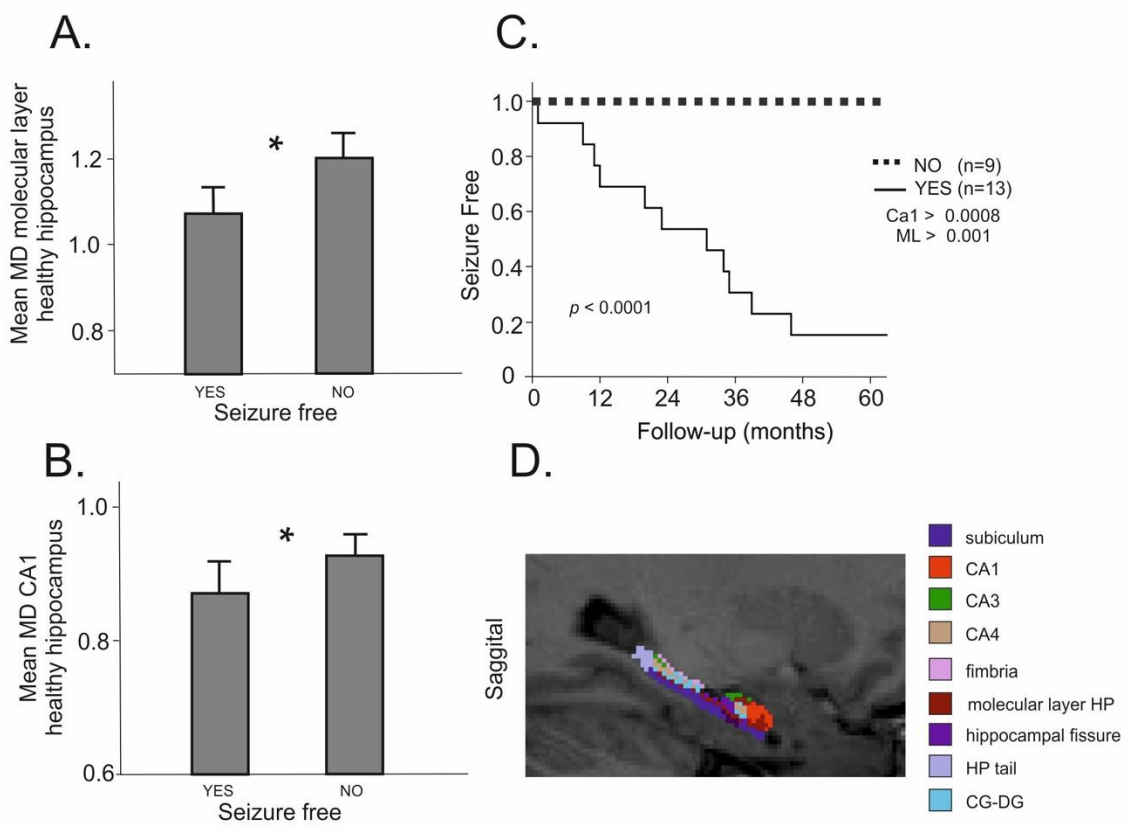


Figure 11. On the left, graphs displaying the mean values (x10^4) of MD for the molecular layer (a) and CA1 (b) for the SF and non-SF groups (*p<0.05). On the top right (c), Kaplan Meier curve comparing the evolution of patients with values of CA1 and Molecular layer MD above and under the cut-off point. Bottom right (d), subfield segmentation of the healthy hippocampus of one of our patients.

4.1.4 Evaluation of the results

This is, to our knowledge, the first attempt to investigate the relation between the contralateral hippocampal subfield microstructure in a set of well-selected unilateral TLE-HS patients, and the long-term seizure outcome after surgery. When compared to controls, our patients demonstrated significant unilateral atrophy in the volumetric assessment in accordance with unilateral TLE-HS. When comparing SF and non-SF groups, our main findings involved changes in the microstructural integrity of the contralateral to the affected side hippocampus.

When we compared SF to non-SF groups, we found significant differences in the contralateral CA1, dentate gyrus and the molecular layer, key structures in epileptogenesis, most likely translating to a more extended epileptogenic network (Bernhardt et al., 2016; Sierra et al., 2015), including these contralateral hippocampal subfields. The bilateral progressive changes in drug-resistant unilateral TLE-HS have long been known, and the relation to seizure relapse has been proposed by others, prompting the importance of early surgery (Bernhardt et al., 2013). In our sample, no differences were found in epilepsy duration. Yet, despite this fact, we still found significant changes in the subfields of the contralateral hippocampus when comparing SF and non-SF groups. Although bilateral changes are commonly related to unilateral TLE-HS, apparently not all the patients with bilateral alterations persist having seizures (Thom et al., 2009). We hypothesized that a critical amount of damage must be suffered to translate into prognosis after surgery. We analyzed which cut-off best differentiated between SF and non-SF patients. In our sample, patients with specific MD values in the molecular layer and the CA1 remained seizure free during long-term follow-up. The patients with less bilateral microstructural disorganization appear to be those who most benefited from surgery.

As previously reported (Miró et al., 2015), patients that suffer from stronger interhemispheric connectivity related to seizure propagation patterns may suffer larger degradation of microstructural integrity, affecting the MD of both hippocampi. Patients with TLE-HS may relapse because of different reasons (Hennessy et al., 2000), but we can use biomarkers assessing the key structures of the TLE-HS network to assess seizure outcome after epilepsy surgery. In line with previous works highlighting the importance of unilaterality (Simon S. Keller et al., 2017) and the involvement of extended networks (Bonilha, Jensen, et al., 2015) in TLE-HS as markers of post-surgical outcome, we put forward the role of microstructural integrity of the contralateral hippocampus as a useful presurgical marker of risk of seizure recurrence. Our results suggest that abnormalities in key regions of the contralateral hippocampus could relate to an extended seizure network, capable of sustaining ictal activity despite the removal of the sclerotic hippocampus in well selected patients with unilateral TLE-HS.

Patients with TLE-HS may show differences in clinical history, structural imaging and pathology suggesting a group of diseases (Malmgren et al., 2012). There are in fact four pathological substrates to HS (Blümcke et al., 2013), that may relate to different epilepsy history and post-surgical outcome; however, presurgical identification of these subtypes remains elusive (Bonilha, Martz, et al., 2012). Using pre-surgical neuroimaging methods to evaluate the TLE-HS network structures have shown promising results in assessing the risk of seizure recurrence after surgery, mainly by measuring abnormalities in areas outside the margins of resection, either of the ipsilateral or the contralateral temporal lobe (Simon S. Keller et al., 2017; Lin et al., 2005). Our findings must be considered in this context, and may provide another framework to asses extended damage on the TLE-HS network.

Our study has several setbacks; mainly, the low number of patients included. As such, our findings need to be replicated in larger series of patients. Also, as we looked the contralateral hippocampus, no tissue sample was available. Most of the patients with TLE-HS who underwent surgery had an improvement in seizure frequency, and we only looked at the patients who were completely SF. On the other hand, we provide evidence linking hippocampal microstructure to long-term seizure freedom on a well-selected sample of TLE-HS patients, having a median follow-up of more than 6 years. We used a freely available software, FreeSurfer, which provides a consistent analysis that can be used in different centers.

In summary, the microstructural anatomy of the hippocampal subfields may relate to surgical outcome in patients with TLE-HS and could contribute to establish a prognosis prior to surgery.

4.2 *STUDY 2: Mapping connectivity fingerprints for presurgical evaluation of temporal lobe epilepsy*

TLE is a heterogenous entity and surgical prognosis varies between patients. Network-based biomarkers have been shown to be altered in TLE patients and hold promise for classifying TLE subtypes and improving pre-surgical prognosis. The aim of the present study is to investigate a network-based biomarker, the DC, on an individual level, and its relation to TLE subtypes and surgical prognosis.

Thirty TLE patients undergoing surgery and 18 healthy controls were included. All patients were followed-up in the same center for a mean time of 6.85 years and classified as SF and non-SF. Using pre-surgical resting state functional MRI, whole brain DC values for patients and controls were calculated. Then, we divided both temporal lobes in three ROIs (mesial, pole and lateral) as these areas are known to behave differently in seizure onset and propagation, delimiting different TLE profiles. The wDC values for the defined ROIs of each individual patient were compared with the healthy group.

After surgery, 14 TLE patients remained SF. As a group, patients had higher DC than controls in both the temporal pole (p<0.05) as well as in the mesial regions (p<0.002) of the affected temporal lobe. When comparing between SF and non-SF patients, a step-wise binary logistic regression model including all the ROIs, showed that having an increased DC of the temporal pole ($p < 0.05$) and of the mesial area ($p < 0.05$) was associated with seizure freedom long-term after surgery.

This study corresponds to: Sala-Padro, J., Miró, J., Rodriguez-Fornells, A., Rifa-Ros, X., Plans, G., Santurino, M., Falip, M., & Càmara, E. (2021). Mapping connectivity fingerprints for presurgical evaluation of temporal lobe epilepsy. BMC Neurology, 21(1)

4.2.1 Background

In the past few years, considering TLE as a brain network disorder has emerged as a conceptual framework hypothesis that has enhanced our comprehension of electrophysiological and imaging observations in individuals with epilepsy. Notably, certain neuroimaging techniques have demonstrated encouraging outcomes in predicting surgical outcomes by scrutinizing brain networks, although they have not yet gained widespread adoption. One such method is rs-fMRI. This technique, which as previously mentioned provides good spatial and temporal information of the epileptic brain, offers promising results in determining the outcome of surgery. In patients with focal epilepsy, a shift from a small world topology network seen on healthy controls to a more ordered network has been described: key findings include higher clustering and lesser integration in the interictal epileptic brain (Van Diessen et al., 2014); also, peaks of local connectivity have been found around the epileptogenic area (Jackson et al., 2017). Furthermore, previous studies have demonstrated how different subtypes of TLE display different network connectivity, measurable by rs-fMRI (Bernhardt et al., 2019; Vaughan et al., 2016). Relating to surgical prognosis, an increase in hubness of the thalamus, by measuring its degree of connectivity, could be associated with relapse after surgery (He et al., 2017). An interesting measure in this growing field that allows the study of local functional synchronization is the DC (Pedersen et al., 2016). This measure considers both the local functional connectivity and the global network alterations, and evaluates the number of strongly correlated links to a given area (Wasserman et al., 1994).

Previous factors relating to poor seizure outcome on TLE are the presence of an abnormality in the pathology specimen after surgery, and its detection with pre-surgical MRI (Téllez-Zenteno et al., 2010); the presence of secondary generalized seizures (A. M. McIntosh et al., 2004) and the involvement of structures outside the mesial temporal lobe in seizure activity, such as the temporal neocortex and the temporal pole (Stéphan

Chabardès et al., 2005), or extratemporal structures (Carmen Barba et al., 2016). The above mentioned factors related to seizure relapse after surgery do also relate to the extent of TLE network disease (Bonilha, Martz, et al., 2012; Simon S Keller et al., 2014). Moreover, different pathological substrates express different network abnormalities (Bernhardt et al., 2019). In this sense, rs-fMRI may be capable of detecting focal brain connectivity and network alterations in the temporal lobe of patients with TLE, which would in turn help in the localization of the epileptogenic area, the definition of the subtype of TLE by assessing the connectivity of different temporal lobe areas and, ultimately, the relationship to prognosis after surgery.

We analyzed a series of patients with TLE undergoing a standard temporal lobe resection, including the information from rs-fMRI analysis for the characterization of the epileptic network, with the goal of predicting the extension of the disease and detecting which patients will benefit most from surgery, through an individual based approach. To this end, we study the DC as a proxy of the focal connectivity within the temporal lobe, accounting from the whole brain functional network and its relationship with the surgical outcome.

4.2.2 Experimental design

4.2.2.1 Patients

We recruited 33 consecutive patients with TLE who underwent a pre-surgical evaluation between 2009 and 2014 and were considered candidates for surgery. Patients underwent a complete work-up with VEEG, standard neuropsychological evaluation, structural MRI, and, in some cases, brain PET. They were diagnosed by epileptologists according to clinical, EEG and imaging criteria. All patients underwent a 3T MRI with a resting state fMRI sequence. We also scanned 18 healthy volunteers with the same protocol, matched for age, gender, handedness and years of education.

We included 30 patients into the study; two patients finally declined surgery and one patient was discarded due to erroneous data acquisition. Of these 30 patients, 17 were women (56.7%) and 15 had left temporal epilepsy. All patients underwent a temporal lobe resection performed by the same surgeon. All pathology samples were processed in our hospital and examined by the same pathologist. After surgery, patients were followed-up on a three to six monthly basis, specifically interrogating for seizure relapse, for a mean time of 82.3 months (6.85 years), with a range of 39 to 118 months.

The normative group was comprised of 18 healthy participants who had no record of neurological illnesses or psychiatric disorders, and were matched for age, gender, handedness and years of education. See Figure 12 for demographic details of the patients and healthy group samples. This study was approved by the Ethical Committee of Hospital of Bellvitge.

4.2.2.2 MRI Data Acquisition

Patients and healthy participants underwent a pre-operative whole-brain structural MRI scans using a 3.0 Tesla Siemens Trio MRI. A 32-channel phased-array head coil system was

used to acquire high-resolution T1-weighted images (slice thickness = 1mm; no gap; number of slices = 240; TR = 2300ms, TE = 3ms, matrix = 256 x 256; FOV = 244mm; voxel size 1x1x1 mm). Resting state fMRI data were collected using a single-shot T2*-weighted gradient-echo EPI sequence (slice thickness = 4 mm; no gap; number ofslices = 32, interleaved order; TR = 2000 ms; TE = 29 ms; flipangle = 80°; matrix = 80 × 80; voxel size = 3 × 3 × 4 mm^3, 110 volumes).

4.2.2.3 Resting-state functional connectivity analysis

Resting-state functional connectivity preprocessing was carried out using the pipelines implemented in Data Processing Assistant for Resting-State fMRI (Chao-Gan et al., 2010) (DPARSF, http://rfmri.org/DPARSF), which is based on Statistical Parametric Mapping (SPM, http://www.fil.ion.ucl.ac.uk/spm) and the toolbox for Data Processing & Analysis of Brain Imaging (Yan et al., 2016) (DPABI, http://rfmri.org/DPABI) running on MATLAB (v17.a, Mathworks, Natick, MA). The preprocessing steps included the exclusion of the first five volumes, slice timing adjustment and realignment for head motion correction. Coregistration between the functional and the structural T1-weighted image, segmentation of the T1-weighted image into different tissues, DARTEL normalization of the functional and structural images to the MNI space using the parameters derived from the segmentation of the T1-weighted image. Subsequently, the covariates including the white matter signal, cerebrospinal fluid signal, Friston 24 motion parameters and polinominal trend, were regressed out from the time series of every voxel. Furthermore, the BOLD signal was filtered in order to reduce potential effects of low-frequency drift and high-frequency physiological noise with a typical temporal bandpass (0.01–0.1 Hz). Finally, the automated anatomical labeling (AAL) template of Tzourio-Mazoyer (Tzourio-Mazoyer et al., 2002) was used to parcellate registered fMRI time series into specific masks and to create the study ROIs for the to-be resected (ipsilateral) temporal lobes. Specifically, the hippocampus,

amygdala and parahippocampus masks were used to define the mesial ROI; the superior and inferior masks of the temporal pole for the temporal pole ROI, and the superior, middle and inferior temporal gyrus masks to define the lateral temporal lobe ROI. These ROIs were selected as these areas are crucial in seizure relapse as well as defining the TLE subtype (Carmen Barba et al., 2016; Stéphan Chabardès et al., 2005).

DC maps, restricted to the defined ROIs, were computed by using the REST toolbox, using a similar approach to that shown by Buckner et al. and Zuo et al. (Buckner et al., 2009; Zuo et al., 2012), as previously described (Di Martino et al., 2013; S. Li et al., 2016; W. Liu et al., 2015). Specifically, first, for each voxel, Pearson's correlation coefficient between the time series of that voxel and all other voxels were calculated, resulting in a connectivity map that represents all other voxels that are correlated with the selected voxel. Then, this correlation map was thresholded at $r > 0.25$. The weighted sum of previous significant positive correlations was calculated to yield the weighted DC at the selected voxel. This process was repeated for each voxel in the brain to produce a voxel-wise whole-brain map of the weighted DC. Then, the individual weighted DC maps were standardized across all voxels by converting to z-scores (Buckner et al., 2009; Van Dijk et al., 2012; Zuo et al., 2012). Finally, before statistical analysis, the individual maps of degree centrality were spatially smoothed using a 8 mm Gaussian kernel

4.2.2.4 Statistical Analyses

Statistical analysis of group demographics, outcome-based classification, and clinical data was performed in SPSS (v.22, SPSS Inc., Chicago, USA). Chi square and two-tailed independent samples Student's t-test were used to describe clinical and sociodemographic differences between groups. Logistic regression was used to classify the patients based on the surgical outcome.

Second level models of voxel-based DC neuroimaging data were performed using SPM12 software (http://www.fil.ion.ucl.ac.uk/spm/). Specifically, in order to explore the normative DC pattern, the individual voxel-wise DC maps of the healthy group were entered into a second-level analysis using a one-sample t-test. Then, in order to identify group differences in voxel-based DC maps, both healthy participants versus TLE patients and SF versus non-SF patients were compared using a two-sample t tests by entering the corresponding voxel-wise wDC maps. Whole-brain level significant results were identified at $p < .005$ and corrected for multiple comparisons at cluster-level ($p < .05$), with a minimum cluster size of 20 contiguous voxels. When examining SF versus non-SF patients, an exploratory threshold was applied ($p < .005$ at voxel-level, uncorrected). This approach is designed to minimize both Type I and Type II errors in a way that facilitates replication of results across future studies (Lieberman et al., 2009). Anatomical and cytoarchitectonic areas were identified using the Automated Anatomical Labeling Atlas (Tzourio-Mazoyer et al., 2002) included in the xjView toolbox (http://www.alivelearn.net/xjview8/).

In order to identify individual regionally specific effects in DC, individual voxel-wise DC maps were compared between each subject and the normative group within the defined ROIs using a two-tailed Crawford's modified *t*-tests (J. Crawford et al., 1998; J.R Crawford et al., 2002), as done in previous studies (Birba et al., 2017; Cervetto et al., 2018). This test is specifically designed to compare a patient to a small sample of control participants (John R. Crawford et al., 2010). Differences were considered statistically significant when $p < .005$. As a next step, for each ROI a binary map of significant DC was calculated, setting all connections below the significant threshold to zero while setting all remaining connections to 1. Then, one binary variable was created to indicate whether any of the voxel of the selected ROI showed significant differences in the DC. This process was repeated for each ROI using MATLAB in-house code (MATLAB R2017a, MathWorks, Natick, MA).

In order to evaluate whether the selected variables could be used to classify the patients based on the surgical outcome, a logistic regression was performed. More specifically, a binary step-wise backward logistic regression (Field, n.d.) between the levels of wDC burden in the different ROI and presence of recurrence during follow-up (i.e. SF versus non-SF) was assessed. The Hosmer-Lemeshow C statistic was used to calculate the goodness of fit of the logistic model (Field, n.d.). Beta parameters (B), Nagelkerke's and Cox-Snell's R^2 values for each model were specified. In addition, the ROC curve was plotted, and the AUC was calculated as a summary statistic for the prognostic model. One way of interpreting the AUC is as the probability that given any 2 participants randomly selected, one who becomes SF and one who relapse after surgery, the model would assign a higher probability of SF than non-SF.

4.2.3 Results

Of the 30 patients, 14 remained completely SF and 16 were non-SF at the end of the follow-up. Comparing SF and non-SF, there were no significant differences in gender (64.3% vs 50%, p=0.431), history of febrile seizures (14.3% vs 25%, p=0.657), history of generalized tonic-clonic seizures (71.8% vs 80%, p=0.590), presence of an MRI lesion (92.9% vs 81.3%, p=0.602) and presence of HS on tissue sample (85.7% vs 65.8%, p=0.399). SF and non-SF patients had the same epilepsy duration (35.16 vs 27.16, p=0.180). For the clinical characteristics of the sample, see Figure 12.

		Global (n=30)	Seizure-free (n=14)	Non-seizure free (n=16)	p	Healthy group (n=18)
Age. Mean y-o (SD)		49.8 (11.1)	50.7 (10.9)	48.9 (11.6)	0.660	49.50 (11.92)
Women n (percentage)		17 (56.7%)	9 (52.9%)	8 (47.1%)	0.484	11 (61%)
Left TLE n (percentage)		15 (50%)	7 (46.7%)	8 (53.3%)	1	
Age of onset of seizures. Mean y-o (SD)		15.2 (11.6)	11.7 (10.1)	18.3 (12.3)	0.124	
Duration of epilepsy Mean years (SD)		27.4 (15.4)	32.7 (13.9)	22.7 (15.5)	0.075	
Febrile seizures n (percentage)		6 (20%)	2 (33.3%)	4 (66.7%)	0.657	
Secondary generalisation n (percentage)		22 (75.9%)	10 (45.5%)	12 (54.5%)	0.682	
ILAE outcome	1		14 (46.6%)			
	2			3 (10%)		
	3			9 (30%)		
	4			2 (6.6%)		
	5			2 (6.6%)		
	6			Nil		
Lesion on MRI n (percentage)		25 (83.3%)	13 (52%)	12 (48%)	0.336	
Pathology	HS	21 (70.0%)	11 (52.4%)	10 (47.6%)	0.440	
	Tumor	1 (3.3%)	1 (100%)	Nil		
	Heterotopia	1 (3.3)	1 (100%)	Nil		
	Normal	5 (16.7%)	Nil	5 (100%)		
	Dual (HS + carvernoma/ tumour)	2 (6.7%)	1 (50%)	1 (50%)		

Figure 12. Data presented as *mean* ± *standard error* and *N (%patient)*. *N* is detailed in individual cells where differing. Age, Age of onset seizure and duration of epilepsy are given in years. The *p*-values refer to independent two-tailed t tests between seizure free and non-seizure free patients. N = number of participants; f = females; ILAE = International League Against Epilepsy; HS = Hippocampal Sclerosis; n.s. = non significant (p>0.05).

We first carried out a voxel-based analysis restricted to the affected TLE regions in the different groups. Indeed, an exploratory analysis to the normative group showed significantly high levels of DC in an extended pattern, highlighting cortical regions in the lateral temporal lobe, especially in the inferior and middle temporal gyri (See Figure 13A). Moreover, the comparison between healthy participants and TLE patients revealed higher DC in both the superior (t=5.02, x=-51, y=9 , z=-15, p<0.009, FDR-corrected at cluster level) and the inferior (t=4.07, x=-54, y=0 , z=-36, p<0.012, FDR-corrected at cluster level) temporal pole structures, as well as in hippocampal regions (t=4.23, x=-24, y=-18 , z=-15, p<0.002, FDR-corrected at cluster level) (See Figure 13B). In addition, after surgery, SF patients presented a reduced level in DC levels in comparison to non-SF patients in parahippocampal regions extended to the temporal pole (x=-21, y=3, z=-27, p<0,001 uncorrected) (See Figure 13C). However, this difference was not statistically significant when correcting for multiple comparisons.

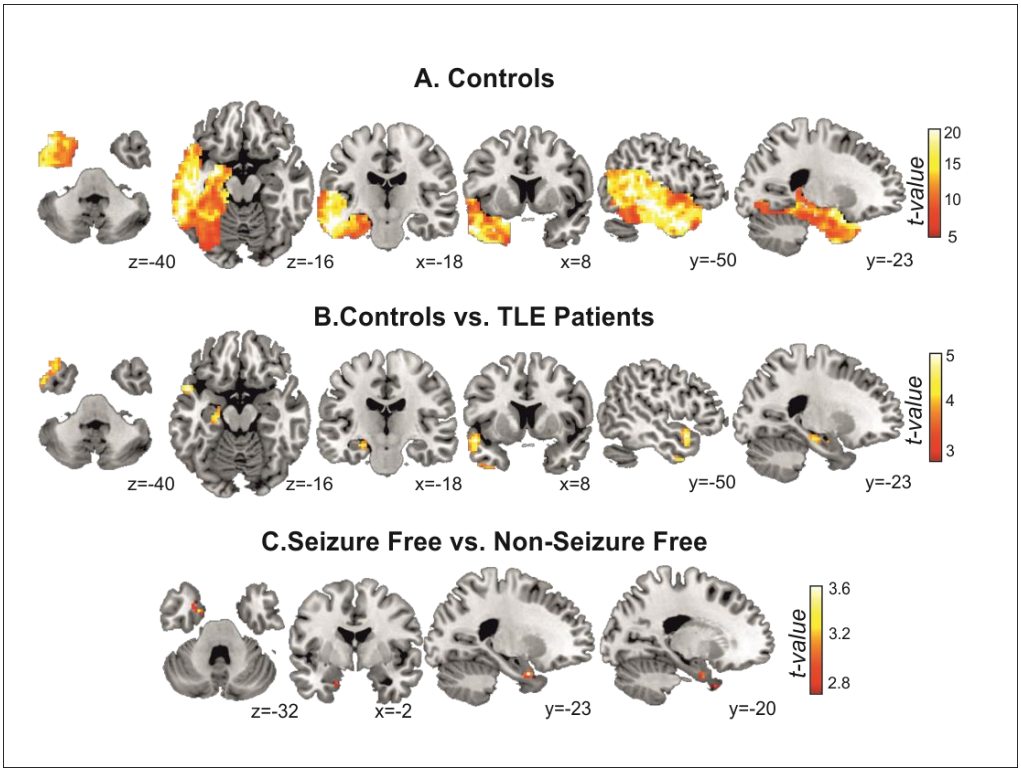


Figure 13. Functional connectivity results at whole-brain level in controls (A) and between group differences (B-C). Slice position is labeled in Montreal Neurological Institute coordinates.

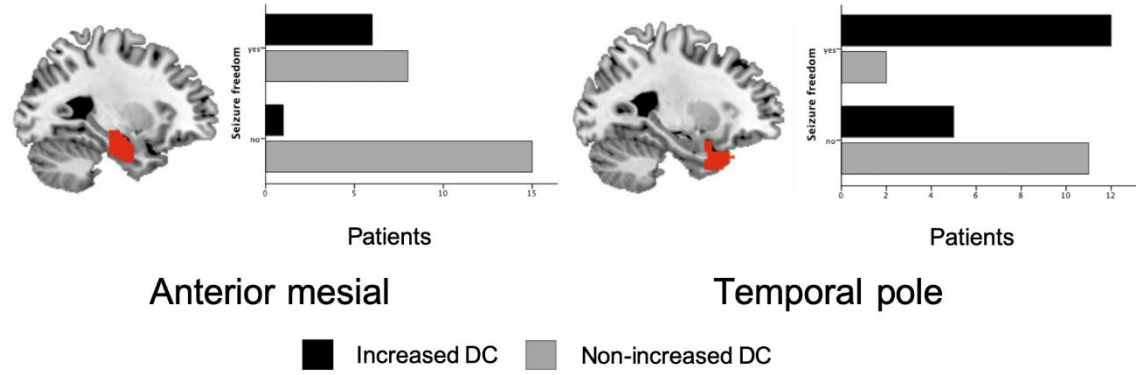
Then, in order to further explore individual differences associated with specific patterns in DC, single subject comparison to the normative group and a logistic regression analysis were investigated. We obtained the individual wDC for the different ROIs described above by comparing each patient with the set of healthy participants. By binarizing the presence or absence of increased DC according to a threshold, we obtained a number of patients with increased DC for each ROI (Figure 19). A majority of patients had an increase of DC of the lateral temporal lobe (96.7% for the ipsilateral side, 93.3% for the contralateral). Approximately half of the patients had increased DC of the temporal pole (56.7% ipsilateral, 46.7% contralateral), and a minority of patients had increased DC of the mesial area (23.3% ipsilateral, 16.8% contralateral) when compared to controls. Due to the fact that the majority of patients in our sample presented HS (70% of the total), we looked at the increase in DC for each ROI in this subgroup of patients (Figure 14).

	HS patients (n=23)		SF (n=12)		Non-SF (n=11)	
	Increased DC	Non-increased DC	Increased DC	Non-increased DC	Increased DC	Non-increased DC
Mesial – ipsilateral	7 (30.4%)	16 (69.6%)	6 (50%)	6 (50%)	1 (9.1%)	10 (90.9%)
Temporal pole – ipsilateral	13 (56.5%)	10 (43.5%)	10 (83.3%)	2 (16.7%)	3 (27.3%)	8 (72.7%)
Lateral temporal - ipsilateral	22 (95.7%)	1 (4.3%)	12 (100%)	Nil	15 (90.9%)	1 (9.1%)
Mesial – contralateral	4 (17.4%)	19 (82.6%)	4 (33.3%)	8 (66.7%)	Nil	11 (100%)
Temporal pole – contralateral	10 (43.5%)	13 (56.5%)	8 (66.7%)	4 (33.3%)	2 (18.2%)	9 (81.8%)
Lateral temporal - contralateral	22 (95.7%)	1 (4.3%)	12 (100%)	Nil	10 (90.9%)	1 (9.1%)

	All patients (n=30)		SF (n=14)		Non-SF (n=16)	
	Increased DC	Non-increased DC	Increased DC	Non-increased DC	Increased DC	Non-increased DC
Mesial – ipsilateral	7 (23.3%)	23 (76.7%)	6 (42.9%)	8 (57.1%)	1 (6.3%)	15 (93.8%)
Temporal pole – ipsilateral	17 (56.7%)	13 (43.3%)	12 (85.7%)	2 (14.3%)	5 (31.3%)	11 (68.8%)
Lateral temporal - ipsilateral	29 (96.7%)	1 (3.3%)	14 (100%)	Nil	15 (93.8%)	1 (6.3%)
Mesial – contralateral	5 (16.7%)	25 (83.3%)	4 (28.6%)	10 (71.4%)	1 (6.3%)	15 (93.8%)
Temporal pole – contralateral	14 (46.7%)	16 (53.3%)	8 (57.1%)	6 (42.9%)	6 (37.5%)	10 (62.5%)
Lateral temporal - contralateral	28 (93.3%)	2 (6.7%)	14 (100%)	Nil	14 (87.5%)	2 (12.5%)

Figure 14 Number of patients with increased wDC for each ROI, for the whole sample and for patients with HS (including patients with dual pathology). Obtained by comparison against controls, a threshold of $p < 0.005$ was applied for considering the wDC of a ROI to be increased. SF – Seizure free. HS – Hippocampal sclerosis.

In a step-wise binary logistic regression model including all the ROIs, having an increased wDC of the temporal pole ($p < 0.05$) and of the mesial area ($p < 0.05$) was associated with seizure freedom long-term after surgery (Figure 15).



Model goodness-of-fit	R^2 Cox-Snell	R^2 Negalkerke	X^2	P-value	Hosmer-Lemeshow
	0.39	0.53	15.19	0.001	0.3
Regressors	B	SE		P-value	
Mesial wDC	-2.88	1.47		0.05	
Temporal pole wDC	-2.91	1.17		0.013	

R2 = coefficient of determination; X2=chi-square; B=coefficient for the predictor; SE=standard error of the coefficient for the predictor; wDC=weighted Degree Centrality

Figure 15. On top, Illustration of the wDC distribution in seizure free and non-seizure free patients in anterior mesial and temporal pole ROIs. Both areas were significantly associated with seizure freedom in the logistic regression model. Below, Model Goodness-of-fit and Regressors Significance of Logistic Regression

The model correctly classified 76.7% of the patients, 92.9% in the SF group, 62.5% in the non-SF group. Finally, the ROC curve was calculated; an AUC of 0.777 was founded (Figure 16).

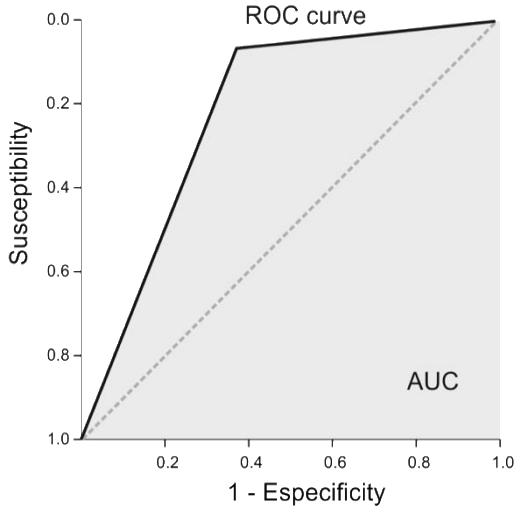


Figure 16. Receiver Operating Characteristic (ROC) curve used to classify the patients based on the surgical outcome and the connectivity parameters extracted from the selected ROIs (Regions of Interest). AUC = Area under the curve.

4.2.4 Evaluation of the results

Our findings reveal crucial network-related biomarkers extracted from individual and quantitative rs-fMRI measures related with surgical outcome in a set of TLE patients with a long-term follow-up. Specifically, at individual level, increases in the local connectivity of key structures within the epileptogenic network in TLE, such as the temporal pole and the mesial area, were related to surgical outcome in our series of patients with TLE undergoing anterior temporal resection. The patient cohort had a mean follow-up of more than 6 years, which means that most of the SF patients will remain cured (De Tisi et al., 2011). This information could be readily available as part of the pre-surgical counselling and evaluation.

DC is a connectivity measure of intrinsic focal connectivity that accounts for the whole brain functional network, highlighting those regions that act as a main stations for information processing (i.e., hubs) that form connections with other segregated networks (Zuo et al., 2012). In our study, we parcellated the temporal lobe into mesial, temporo-lateral and temporo-polar areas. These three regions are known to be implicated in the genesis of temporal lobe seizures and, moreover, relate with both TLE subtypes and surgical prognosis, as highlighted in previous studies (Stéphan Chabardès et al., 2005; Hennessy et al., 2000). In order to focus on individual based data, we used a voxel-to-voxel approach and compared each patient with a set of healthy participants. We found that increased DC of the temporo-polar and the antero-mesial regions were related with the odds of remaining SF long-term after the surgery.

Although there is a great overlap between the different connectivity patterns associated with TLE, there is some degree of differentiation. Patients with TLE display increased local connectivity, consistently reported in the epileptogenic area, with decreased long-range connectivity (Englot et al., 2016). The measurement of peaks of local connectivity has been related to the epileptogenic area, and its resection has translated into improvement of whole

brain network topology (Jackson et al., 2017). Outside the temporal lobe, an increase in connectivity of the thalamus related to poorer seizure prognosis in patients with TLE, probably relating to a widespread network disease (He et al., 2017). Conversely, reductions in long range connectivity measures with increases in local connectivity, when confined to the resected to be temporal lobe, were related to surgical prognosis (Larivière et al., 2020). Using a measure of focal voxel-wise connectivity, we investigated the DC in the to-be-resected temporal lobe, parcellated in three areas that both relate to the subtype of TLE and are known to have implications in surgical prognosis. Specifically, an increased DC related to improved surgical prognosis, which may be attributed to a peak of strong local connectivity, i.e., a marker of the epileptogenicity of the region, and therefore indicate the appropriateness of its resection. TLE with or without HS comprises different entities, with variable extra-mesial involvement, and different patterns of seizure relapse after surgery (Bonilha, Martz, et al., 2012). Notwithstanding, measuring local increased connectivity on an individual level could be a helpful biomarker in evaluating the connectivity within the to-be-resected temporal lobe, which evidence suggest it to be relevant in surgical prognosis.

In our sample, patients with an increase in temporo-polar DC compared with controls had higher odds of becoming seizure-free. The temporo-polar cortex is known to be involved in seizures arising from the temporal lobe (Stéphan Chabardès et al., 2005). In a study using independent component analysis from rs-fMRI data that compared patients with mesial TLE due to different etiologies with healthy controls, found that those patients with mesial TLE patients demonstrated increased connectivity between the temporal pole and the post-central gyrus (Maneshi et al., 2014). Our sample consists mainly of patients with clinical and EEG findings suggestive of mesial TLE, and the majority of patients had HS on the tissue sample (Figure 17). However, not all patients with HS had increased tem-

poro-polar DC, and the same proportion of patients without HS also had increased temporo-polar DC (Figure 19). In a study using intracranial recordings, a key role of the temporal pole cortex was reported in TLE seizures, in close relation with the hippocampus (Stéphan Chabardès et al., 2005). Patients with quicker involvement of the temporal pole had better surgical outcome than those with late involvement. We therefore hypothesized that, due to the fact that the temporo-polar area is a key region in TLE, its increased DC represents a network abnormality that may relate to a better surgical prognosis.

The other major finding is that an increased mesial DC was also related to a better prognosis, with only one patient relapsing after surgery. This mesial DC increase was seen only in our patients with HS on pathology (Figure 19). Previous reports using rs-fMRI have shown that patients with HS displayed increased connectivity of the mesial structures, and this is distinctive from non-lesional MRI TLE patients (Vaughan et al., 2016). In our sample, all the patients with HS and increased connectivity remained seizure free except one. Interestingly, the relapsed patient that demonstrated an increased DC on the mesial structures was an individual with dual pathology. As HS may be a heterogenous group of diseases (Malmgren et al., 2012), identifying markers of good surgical response is of paramount interest for the clinician; we related our finding of increased DC to HS with better prognosis. This finding needs to be reported with longer series in order to study whether an increased DC of the anterior mesial area in patients with HS represents a subgroup of patients with better prognosis.

Assessing the extension of the epileptogenic zone and identifying the subtype of TLE is crucial to decide the candidacy for a temporal lobe resection, the need of intracranial evaluation and establishing a prognosis. One of the most important factors for a surgical procedure in TLE is having a lesion on MRI. However, there is still a proportion of patients with lesions on MRI that relapse, as well as patients with normal MRI that benefit from

surgery (Jehi, 2018). Beyond the MRI being lesional or not lesional, studies using PET and SPECT have rendered different patterns that relate to different prognosis, helping decision making (M. L. Bell et al., 2009; Carne et al., 2004). From our study, the role of rs-fMRI could help identify key network abnormalities that may improve the assessment of prognosis before the surgery.

Several considerations form our study have to be taken. First, as we studied our own cohort of TLE surgical patients, our sample consists mostly of MRI lesional patients, as patients with HS formed a large proportion of the total sample. While the use of rs-fMRI would not change decision making, using our findings as reported could provide extra information on prognosis at the individual level. We report few TLE patients with normal MRI and normal pathology, which are presumably the ones in need of more pre-surgical tools to help decision-making. Still, in our small sample of normal MRI and normal pathology, the findings reported were valid (Figure 17). In any case, longer series are needed to validate the usefulness of rs-fMRI.

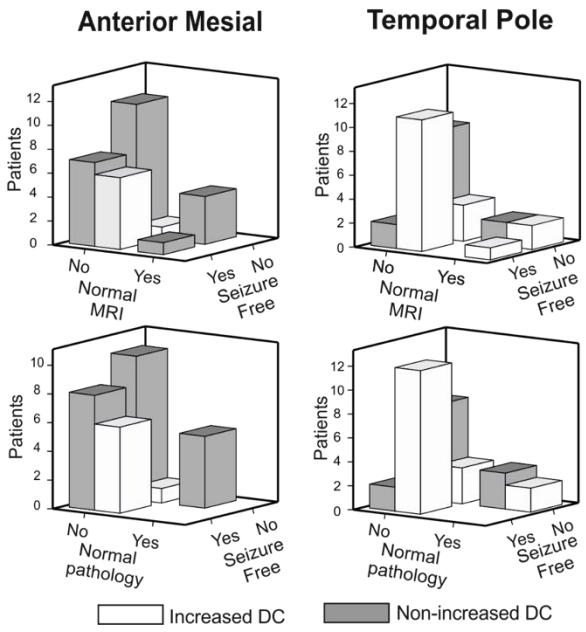


Figure 17. Distribution of patients with normal MRI and normal pathology, relation with seizure freedom and increased wDC. Left panel represents the distribution in the anterior mesial region; right panel shows the temporal pole of the epileptogenic brain size.

As mentioned, TLE is a heterogeneous disease, with many factors influencing the surgical outcome. We strove to control for clinical factors in SF and non-SF groups (no differences on history of febrile convulsions or generalized tonic-clonic seizures), and we report a similar number of patients with lesional MRI and HS on pathology on both SF and non-SF groups. Finally, due to the fact that all patients underwent anterior temporal lobectomies performed by the same surgeon, we did not control the tissue resected. Further studies should account for the resected tissue after the surgical procedure, by measuring the DC of the actual resected area and investigating network changes after the resection.

Elucidating the neurobiology of the different profiles within TLE is of paramount relevance for the stratification of surgical outcomes. Fingerprinting brain connectivity biomarkers of the to-be-resected temporal areas could contribute to the establishment of a prognosis prior to surgery and provide more promising personalized healthcare.

4.3 *STUDY 3: Verbal Learning and Longitudinal Hippocampal Network Connectivity in Temporal Lobe Epilepsy Surgery*

Learning new verbal information can be impaired in 20-40% of patients after mesial temporal lobe resection. In recent years, understanding epilepsy as a brain network disease, and investigating the relationship between large-scale resting networks and cognition has led to several advances. Aligned studies suggest that it is the integrity of the hippocampal connectivity with these large-scale networks what is relevant for cognition, with evidence showing a functional and structural heterogeneity along the long axis hippocampus bilaterally. Our aim is to examine whether pre-operative resting-state connectivity along the long hippocampal axis is associated with verbal learning decline after anterior temporal lobe resection.

Thirty-one patients with epilepsy who underwent an anterior temporal lobe resection were pre-surgically scanned at 3-tesla, and pre/post-surgery evaluated for learning deficits using the RAVLT. Eighteen controls matched by age, gender and handedness were also scanned and evaluated with the RAVLT. We studied the functional connectivity along the (anterior/posterior) long axis hippocampal subregions and resting-state functionally defined brain networks involved in learning (DMN, DAN & EXE). Functional connectivity differences between the two groups of patients (learning intact or with learning decline) and controls were investigated with MANOVA and discriminant analysis.

There were significant differences in the pattern of hippocampal connectivity among the groups. Regarding the anterior connectivity hippocampal pattern, our data showed an increase of connectivity in the pathological side with the DAN ($p=0.011$) and the EXE ($p=0.008$) when learning-decline vs. learning-intact patients were compared. Moreover, the non-pathological side showed an increase in the anterior connectivity pattern with the DAN

(p=0.027) between learning-decline vs. learning-intact patients. In contrast, the posterior hippocampus showed a reduction of connectivity in the learning-decline patients with the DMN, both in the pathological (p=0.004) and the non-pathological sides (p=0.036). Finally, the discriminant analysis based on the pre-operative connectivity pattern significantly differentiated the learning-decline patients from the other groups (p=0.019).

Our findings suggest bilateral connectivity disruptions along the longitudinal axis of the hippocampi with resting-state networks, which could be key in helping to identify those patients at risk of verbal learning decline after epilepsy surgery.

This study corresponds to: Sala-Padro, J., Gifreu-Fraixino, A., Miró, J., Rodriguez-Fornells, A., Rico, I., Plans, G., Santurino, M., Falip, M., & Càmara, E. (2022). Verbal Learning and Longitudinal Hippocampal Network Connectivity in Temporal Lobe Epilepsy Surgery. Frontiers in Neurology, 13(June), 1–12. doi: 10.3389/fneur.2022.854313

4.3.1 Background

Patients with TLE are at risk of verbal learning impairment after surgery; assessed through the RAVLT, up to 20-40% of patients can be impaired, as the resection affects mostly the anterior part of the mesial temporal lobe, including the hippocampus, which is fundamental in memory and learning (S. Baxendale, 1998).

The hemispherical side of the surgery, the resection extent, the verbal learning skills prior to surgery, or the age of seizure's onset are factors modulating the risk of suffering this decline (B. Bell et al., 2013). However, not all patients with epilepsy show verbal learning deficits postoperatively, in that some patients perform similarly before and after surgery (Martin et al., 1998). Such findings indicate that some patients have the neuroplastic potential to compensate for the resected area, leading to post-operative preservation.

Different neural mechanisms might explain the individual differences in the maintenance of verbal learning despite seizure-related and resection-related damage. Evidence suggests that these mechanisms may lead to changes in the connectivity pattern of different neural networks to cope with the impact of pathology and epileptic seizures. In this sense, disruption of neural networks in TLE patients have been reported to be related with cognitive functions (G. Doucet et al., 2013). More specifically, a decrease in functional connectivity between the hippocampus and the posterior cingulate cortex, a key hub in the DMN, has been consistently reported in relation to verbal memory deficits (G. Doucet et al., 2013; Mccormick et al., 2013).

Both up-regulation and down-regulation of functional connectivity can be found in patients with TLE (Bonilha, Nesland, et al., 2012). Using graph theory measures, a decrease in nodal efficiency of the left hippocampus was related to impairment of verbal memory in patients with left TLE, whereas increases in nodal efficiency of the inferior frontal gyrus and the supplementary motor area were correlated to semantic and phonological fluency in

patients with right TLE (Roger et al., 2020). For those undergoing surgery, down-regulation ipsilateral to the affected side coupled with concurrent contralateral up-regulation of functional connectivity has been associated with a preserved cognitive outcome after the procedure (Su et al., 2015). In this vein, higher connectivity of the pathological hippocampus with the posterior cingulate was associated with a high risk of post-surgical decline, whereas the connectivity of the contralateral hippocampus to the posterior cingulate related to less risk of decline (Mccormick et al., 2013). Finally, another study using graph-theory measures (efficiency, integration and centrality), reported an enhanced integration of the contralateral hippocampus to be predictive of cognitive preservation after surgery, and increased connectivity in the inferior frontal gyrus was related with preserved performance of language tasks (G. E. Doucet et al., 2015). In summary, evidence suggest a bilateral reorganization of network connectivity involving both hippocampi and extrahippocampal structures in patients with TLE, that is related to verbal memory and post-surgical decline.

Further evidence also showed relevant changes along the longitudinal axis in the to-be resected hippocampus. A shift in hippocampal activation during an encoding task from the anterior regions towards more post-surgically preserved posterior regions of the to-be resected hippocampus conferred protection against verbal memory impairment after surgery (Bonelli et al., 2013). This would suggest some level of reorganization also along the long hippocampal axis associated with decline after resection, yet their underlying neural mechanisms remain poorly understood. In terms of the functional heterogeneity of the anterior and posterior hippocampus and their involvement in cognitive functions, aligned animal and human neuroimaging studies suggest that cognitive domains superimpose according to a functional gradient along the longitudinal axis (Strange et al., 2014), which also exhibits differences in functional connectivity in TLE patients (Voets et al., 2014).

Noteworthy, evidence from recent years indicate that it is the integration of the hippocampi with bilateral, large scale networks what is relevant for cognitive function (Chand et al., 2018; Persson et al., 2018); these large-scale networks can be measured reliably at rest (Damoiseaux et al., 2006), providing a useful tool for connectivity analysis. Network disruption has proven to be the key underlying some neurological deficits (Boes et al., 2015), and might be a suitable candidate to predict neuropsychological impairment that is less dependent on lesions in TLE (Rayner et al., 2019). Moreover, differences in connectivity and functionality along the longitudinal axis of the hippocampal formation have revealed two systems (Ranganath et al., 2012). Despite both systems playing a role in learning and retrieval tasks, it has been hypothesized that they differ in the connectivity with different large-scale networks, with the anterior portion closely connected to the DAN and EXE and related to encoding of external stimuli, while the posterior portion is more closely connected with the DMN and related to memory retrieval and internal sources of information (Kim, 2015). These large-scale networks involve mainly fronto-parietal areas, and their relation to cognitive functions has long been stablished (Fornito et al., 2012; Smith et al., 2009).

Regarding the cognitive function, we have investigated the impact of the bilateral functional connectivity along the longitudinal axis of the hippocampus with these large-scale networks and how this could relate to the risk of verbal learning impairment seen in post-surgical epilepsy patients. Of note, the above-mentioned studies in TLE patients evaluated the connectivity of the hippocampus as a whole. However, the longitudinal axis of the hippocampus displays differences in connectivity with large scale networks, and this fact relates to the cognitive functions supported (Grady, 2019; Kim, 2015; Ranganath et al., 2012; Voets et al., 2014). More specifically, we focused on verbal learning abilities that could be impaired after surgery assessed through RAVLT, since previous studies have demonstrated deficits

in patients undergoing temporal lobe surgery (S. Baxendale, 1998). Verbal learning requires different cognitive processes to be intact in order to be performed correctly. The RAVLT evaluates verbal learning requiring both retrieval (Rouder et al., 2001) and encoding processes, as well as attentional shifting strategies (Hashimoto et al., 2004) and executive functioning (Perri et al., 2013). These processes that are involved in verbal learning require the activation of both the anterior and posterior hippocampal formation.

Specifically, we compared the pre-surgical connectivity of both hippocampi between patients who had an impairment in the RAVLT learning domain after surgery with those who had not. We hypothesized that functional connectivity of the anterior and posterior hippocampal formation with DMN, EXE and DAN differ in patients with verbal learning impairment after mesial temporal resections already before epilepsy surgery. We also analyzed this connectivity for the hippocampus as a whole, to investigate whether the anterior/posterior division provided further information on these patients. This study offers the possibility to identify biomarkers that predict the prognosis of the surgical outcome, which may be key for the pre-surgical planning.

4.3.2 Experimental design

4.3.2.1 Participants

We included 31 consecutive patients who underwent an anterior temporal lobe resection for epilepsy surgery in the period 2009-2014. All patients were operated by the same neurosurgical team, and all the tissue was analyzed in the neuropathology department of our hospital. We also scanned 18 controls matched by age (T-student=0.15, p=0.516), gender (X^2=0.44, p=0.834) and handedness (X^2=0.016, p=0.9).

From the 31 patients, 18 were women (58.1%), with a median age of 49 years old (range 44 years). Sixteen patients (51.6%) had a left mesial temporal lobe resection, and 24 (77.4%) had signs of hippocampal sclerosis on the tissue sample. After surgery, 14 patients (45.2%) remained completely seizure free during follow-up (median time of follow-up 76 months, range 55 months). There was no difference among patient's groups in terms of seizure freedom after surgery (learning decline 45.5% of seizure freedom vs learning intact 60% of seizure freedom, X^2=0.61, p=0.436) See Table 1 for demographic details of the patients and healthy control group samples. The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained for every participant, and the study was approved by the Clinical Research Ethics Committee of Bellvitge University Hospital.

4.3.2.2 Verbal learning assessment

All patients were assessed using the RAVLT (Schoenberg et al., 2006) before and after surgery for evaluation of cognitive functioning. The stimuli that were presented in each of the evaluation were different. The test was performed in a median time of 9 months (range 5 to 19 months) before surgery, while the evaluation after surgery was performed in a median time of 6 months (range 4 to 12 months). Between both tests the span of time was a median of 17 months (range 11 to 28 months). In this task, the evaluator reads 15 different

words to the patients. In an immediate recall test, patients are asked to repeat after each list all the words they could remember, regardless of the order in which they were presented by the experimenter. This task was repeated five times in a row. The final performance in the five lists was recollected as the absolute number of words remembered in the last run for each subject. This final score as an absolute number of words was used as a measure of verbal learning performance. Then, this score was transformed into standardized values using normative data from Hispanic cohorts, corrected for age, gender and educational level (Pontón et al., 1996).

4.3.2.3 MRI Data Acquisition

Patients underwent a pre-operative whole-brain structural MRI scans using a 3.0 Tesla Siemens Trio MRI. A 32-channel phased-array head coil system was used to acquire high-resolution T1-weighted images (slice thickness = 1mm; no gap; number of slices = 240; TR = 2300ms, TE = 3ms, matrix = 256 x 256; FOV = 244mm; voxel size 1x1x1 mm). Resting state fMRI data were collected using a single-shot T2*-weighted gradient-echo EPI sequence (slice thickness = 4 mm; no gap; number of slices = 32, interleaved order; TR = 2000 ms; TE = 29 ms; flipangle = 80°; matrix = 80 × 80; voxel size = 3 × 3 × 4 mm^3, 110 volumes). During the resting state, participants were instructed to keep still with the eyes closed but not fall asleep, and to not focus on any thoughts, as far as possible. Healthy participants underwent the same neuroimaging protocol.

4.3.2.4 Hippocampal volumes

Total hippocampal volumes were segmented from a fully automated pipeline for hippocampal subfields including the automated cortical parcellation and subcortical recon-all tools implemented in FreeSurfer 6.0 (http://surfer.nmr.mgh.harvard.edu/). The technical details have been described previously (Sala-Padro et al., 2020). To adjust

for differences in head size, all volumes were normalized to ICV by dividing by intracranial ICV calculated using FreeSurfer. Finally, all generated hippocampal images were visually inspected to ensure there were no technical failures or mislabeling.

4.3.2.5 Resting-state functional connectivity analysis

ICA was used to delineate spatially independent and temporally coherent patterns of functional brain connectivity in the resting state DMN, EXE and DAN.

Individual functional data pre-processing was carried out using Statistical Parameter Mapping software (SPM12, Welcome Department of Imaging Neuroscience, University College, London, UK, http://www.fil.ion.ucl.ac.uk/spm/) following the standard protocol. The preprocessing of the data included realignment, co-registration between the structural T1 and their respective mean functional image, normalization, spatial smoothing (FWHM 8 mm). Then, to extract the different functional networks by means of component analysis, we used the GIFT software (http://icatb.sourceforge.net/) (Calhoun et al., 2001). Thus, smoothed data of all participants, combining patients and healthy controls, were temporally concatenated into a 4D time series and then decomposed into different temporal dimensions using principal component analysis and constrained to 20 components, to be then analyzed with the Infomax algorithm (A. J. Bell et al., 1995).

ICA was performed 100 times and the results were clustered by GIFT toolbox ICASSO min cluster size of 15 and max of 20 (number of runs) and RandInit and Bootstrap were selected. Finally, there was a back-reconstruction process from the group ICA components estimated to the individual activation values for each participant of the different groups and for each component. This process allowed the estimation of different spatial component maps for each individual in terms of voxel-wise z scores. Then, within the obtained ICA

networks in the group of participants, we identified the common resting-state intrinsic connectivity networks by visual inspection, and among them, we selected the networks of interest. Two independent authors (JS, EC) separately reviewed the 20 components from the ICA, and after visual inspection and in accordance to published data (Smith et al., 2009) identified the DMN, DAN and EXE networks.

For the resting-state fMRI data, the group-level EXE, DAN and DMN maps were identified using a one-sample t test (including both positive and negative effects) after entering the individual resting-state maps for both patients and controls. In order to define the group level resting-state connectivity map for each network, results were reported using a threshold of $p < 0.05$ with Family-wise error correction for multiple comparisons at whole brain level. The maxima of suprathreshold regions were localized by rendering them onto a normalized T1 structural MNI reference brain.

Then, for each participant, the whole hippocampi and four seed regions (left/right, anterior/posterior) were defined based on hippocampal delineations and, the z-scores across all voxels within the selected ROI were averaged in the different networks at $p < 0.05$ (uncorrected), representing the functional connectivity magnitude between the hippocampal ROI and each network.

The different ROIs were delineated following the same approach described previously (Poppenk et al., 2013). Specifically, the segmentation of hippocampi was performed according to the Anatomical Automatic Labeling brain atlas (Tzourio-Mazoyer et al., 2002). The anterior and posterior subdivision was made just posterior of the uncal apex ranged from y=-2 to -18 (anterior) and from y=-24 to -42 (posterior) in MNI coordinates. Between the anterior and posterior part of the hippocampal division, a gap of 4 mm was left in order to

reduce inter-regional effects because of smoothing and registration errors (Persson et al., 2018).

4.3.2.6 Statistical analysis

The statistical analysis was performed with SPSS (v.25, SPSS Inc., Chicago, USA). First, in order to identify those patients that presented cognitive impairment after surgery, we compared the scores of verbal learning task before and after surgery on an individual level. Specifically, we classified those patients who had a decline of two standard deviation of the normalized score or more in verbal learning as learning-decline, and the patients who showed a decline below two standard deviation or no decline after surgery as learning-intact. In order to define significant decline we applied the two standard deviation cut-off as it is a highly stringent according to previous reports (S. Baxendale et al., 2005)

Second, statistical analysis of groups (learning-decline vs. learning-intact vs controls) demographics, clinical data including learning scores, and total hippocampal volume was performed. Log linear analysis and ANOVA were used to describe sociodemographic, clinical and hippocampal volume differences. If there was a significant difference among groups, a post-hoc univariate analysis using Bonferroni's correction was performed. When comparisons were made between learning-decline and learning-intact groups, T-student and Chi-square tests were performed to assess statistical significance.

Third, to investigate differences in the hippocampal longitudinal axis connectivity according to the neuropsychological performance, a MANOVA with Tukey post-hoc test was performed, considering the three groups (controls, learning-decline and learning-intact). First, Box's test was performed to check equality of covariance. Then, overall significance of the MANOVA test was assessed with Pillai's trace. This was followed by univariate analysis with Tukey correction. Finally, in order to corroborate which hippocampal longitudinal axis connectivity with the different networks outcomes could be used to correctly classify

the different groups based on the surgical cognitive deficits, a linear discriminant analysis was performed, and a ROC curve was configured for the variables showing significant differences on the discriminant function. These analyses were performed for the anterior/posterior division and for the whole hippocampus separately.

4.3.3 Results

After classifying the patients according to the cognitive deficits postoperatively in the verbal learning task, 20 patients were learning-intact and 11 suffered a learning-decline after surgery. Preoperatively, no differences were found between groups on the RAVLT. After surgery, a significant difference was found when comparing controls and learning-intact patients with the patients of the learning-decline group (F=7.09, p=0.002). See Figure 18 for sociodemographic and clinical differences.

	Learning decline N=11	Learning intact N=20	Controls N=18	p
Age (years)	47 (7.5)	51.7 (12.1)	49.5 (11.9)	0.516
Sex (female)	6 (54.5%)	12 (60%)	11 (61.1%)	0.937
Age at onset (years)	18.1 (13.3)	12.6 (10.4)		0.207
Left resection (participants)	8 (72.7%)	8 (40%)		0.081
HS (participants)	8 (72.7%)	16 (80%)		0.643
Seizure-free* (participants)	6 (54.5%)	8 (40%)		0.477
Post-surgical follow-up **	86 (50)	93.5 (55)		0.919
Hipp volume (cm^3)				
Pathological	2.77 (0.5)	2.8 (0.5)	3.33 (0.3)	<0.001
Contralateral (Participants)	3.41 (0.2)	3.52 (0.2)	3.41 (0.3)	0.154
RAVLT pre	12.2 (2.2)	11.1 (2.2)	11.7 (2.4)	0.472
RAVLT post	8.2 (1.2)	11.5 (2.7)	11.7 (2.8)	0.002

Figure 18. Sociodemographic and clinical characteristics of Temporal Lobe Epilepsy patients and controls. Data presented as number (percentage). N=number of participants; HS=Hippocampal Sclerosis; Hipp= Hippocampus; RAVLT= Rey Auditory Verbal learning Test.

*At the end of follow-up

**In months, median (range). Significance assessed with the Mann-Whitney test

Global hippocampal volume differences among the three groups were investigated. Comparing the pre-surgical hippocampal volume, both the learning-intact and learning-decline groups had a decreased hippocampal volume for the to-be resected side when compared with the controls ($F=9.79$, $p<0.001$) with no significant differences between the two groups of patients on the post-hoc analysis. Also, there were not significant differences in the hippocampal volume among groups on the first-level ANOVA for the contralateral side ($F=1.97$, $p=0.154$).

Group-level EXE, DAN and DMN map of patients and controls is shown in Figure 19. Regions with a positive coupling corresponded to areas typically reported in the literature as part of the EXE (i.e., dorsal and anterior frontal areas), DAN (i.e. superior parietal and dorsolateral frontal areas) and DMN (i.e. posterior cingulate-precuneus and orbitofrontal regions).

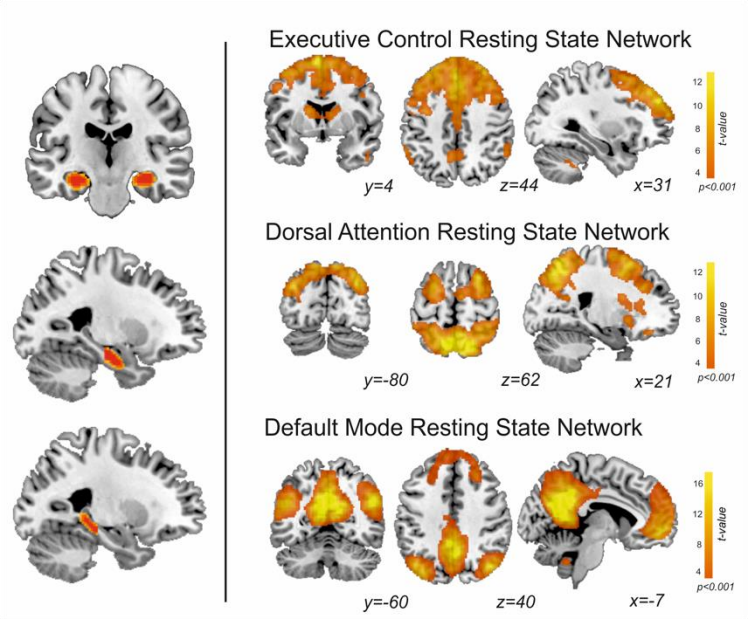


Figure 19. On the left, segmentation of both hippocampi, and division along the anterior-posterior axis. On the right, the resting state networks obtained in this study from patients and controls.

We first performed MANOVA among the three groups (learning decline, learning intact and controls) comparing the connectivity of the whole hippocampus with the DMN, EXE and DAN networks. Using Pillai's trace, there was a significant difference of the pattern of hippocampal connectivity among the groups [V = 0.95, F (12, 84) = 6.3, $p < 0.001$]. See Figure 20 for details.

		L-D	L-I	Controls	p
Pathological side					
Anterior hippocampus	DMN	1.2 (2.6)	0.009 (2.2)	1 (2.1)	n.s.
	DAN	1.56 (2.5)	-0.86 (2.3)	-0.37 (1.8)	<0.05
	EXE	2.6 (3.5)	-0.61 (2.6)	1.66 (3.4)	n.s.
Posterior hippocampus	DMN	-1.27 (2.1)	0.44 (1.9)	0.96 (1)	<0.05
	DAN	0.57 (2.6)	0.41 (2.3)	0.12 (2.5)	n.s.
	EXE	0.13 (3.2)	-0.64 (2.1)	-1.1 (2.3)	n.s.
Whole hippocampus	DMN	-0.38 (2.08)	-0.86 (1.96)	3.64 (2.1)	<0.05
	DAN	0.98 (0.98)	0.13 (1.8)	-1.15 (2)	<0.05
	EXE	0.1 (2.02)	-.094 (1.96)	-0.13 (2.4)	n.s.
Healthy side					
Anterior hippocampus	DMN	-0.15 (2.8)	0.29 (2.1)	1.31 (1.8)	<0.05
	DAN	2.1 (2.7)	-0.11 (2.2)	1.18 (2.3)	n.s.
	EXE	1.92 (1.8)	0.87 (3.4)	1.4 (3.7)	<0.05
Posterior hippocampus	DMN	-0.04 (2.3)	1.61 (1.7)	1 (1.4)	<0.05
	DAN	0.74 (2.6)	0.44 (2)	0.81 (2)	n.s.
	EXE	-0.002 (2.7)	-1.05 (2.4)	-0.1 (1.9)	n.s.
Whole hippocampus	DMN	-0.81 (1.09)	0.57 (1.42)	4.84 (2.05)	<0.05
	DAN	0.82 (2.54)	0.46 (2.18)	-0.82 (2.05)	n.s.
	EXE	0.71 (2.1)	-0.56 (2.34)	0.12 (1.89)	n.s.

Figure 20. Connectivity analysis results for each group. Data represents the z-scores across all values of the hippocampal subregions averaged in the different networks, presented as mean (standard deviation).

L-D=learning decline; L-I=learning intact; DMN=Default Mode Network; DAN=Dorsal Attention Network; EXE=Executive Network. Significance was assessed with a MANOVA test as described previously.

For the whole hippocampus analysis, there were differences on the DMN connectivity with both the healthy side [F (2, 46) = 51.2, $p < 0.001$] and the pathological side [F(2, 46) = 30, $p < 0.001$], and on the DAN connectivity with the pathological hippocampus [F(2, 46) = 5.54, $p < 0.007$]. The post-hoc analysis revealed a significant decrease in global hippocampal connectivity bilaterally with the DMN for patients (both learning-decline and learning intact) when compared to controls ($p<0.0001$, Tukey corrected). The post-hoc analysis on the pathological hippocampus with the DAN connectivity revealed an increase in connectivity for the patients with learning decline compared to controls ($p=0.007$, Tukey corrected).

We then performed a MANOVA among the three groups comparing the connectivity of the anterior and posterior hippocampi with the DMN, EXE and DAN networks. Using Pillai's trace, there was a significant difference of the pattern of hippocampal connectivity among the groups [V = 0.73, F (24, 72) = 1.72, $p = 0.041$]. See Figure 21 for comparison results.

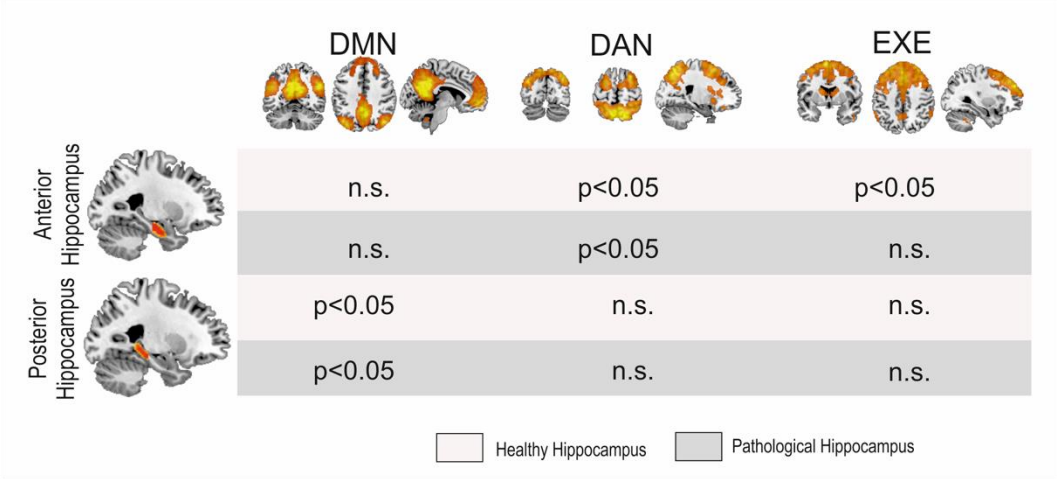


Figure 21. MANOVA results. Significant differences were found among groups in the posterior hippocampi with the DMN and the anterior hippocampi with the DAN and the EXE, although for the latter only with the pathological side.

DMN= Default Mode Network; DAN= Dorsal Attentional Network; EXE= Executive Control Network.

With regard to the anterior connectivity hippocampal pattern, separate univariate tests revealed a significant difference in the pathological side with the DAN [F (2, 46) = 4.75, p = 0.013] and with the EXE [F (2, 46) = 5.65, p = 0.006]. Posterior post-hoc analyses showed that these patterns reflect a significant increase in connectivity in the DAN (p = 0.011, Tukey corrected) and in the EXE (p = 0.008, Tukey corrected) between learning-decline vs. learning-intact patients. Moreover, the non-pathological side showed a significant difference in the anterior connectivity pattern with the DAN [F (2, 46) = 3.90, p = 0.027], in which further post-doc analyses indicated a significant increase of connectivity (p = 0.022, Tukey corrected) between learning-decline vs. learning-intact patients.

In contrast, the posterior connectivity hippocampal pattern only showed differences with the DMN, both in the pathological [F (2, 46) = 6.15, p = 0.004], and the non-pathological [F (2, 46) = 3.57, p = 0.036] side. On post-hoc analysis, the connectivity of the posterior pathological hippocampus with the DMN was significantly decreased in learning-decline patients when compared with learning-intact patients (p = 0.029, Tukey corrected) and controls (p = 0.003, Tukey corrected). The connectivity of the posterior non-pathological hippocampus was also significantly decreased in learning-decline patients compared to learning-intact patients (p = 0.027, Tukey corrected), but there was no difference with controls. See Figure 22 for results.

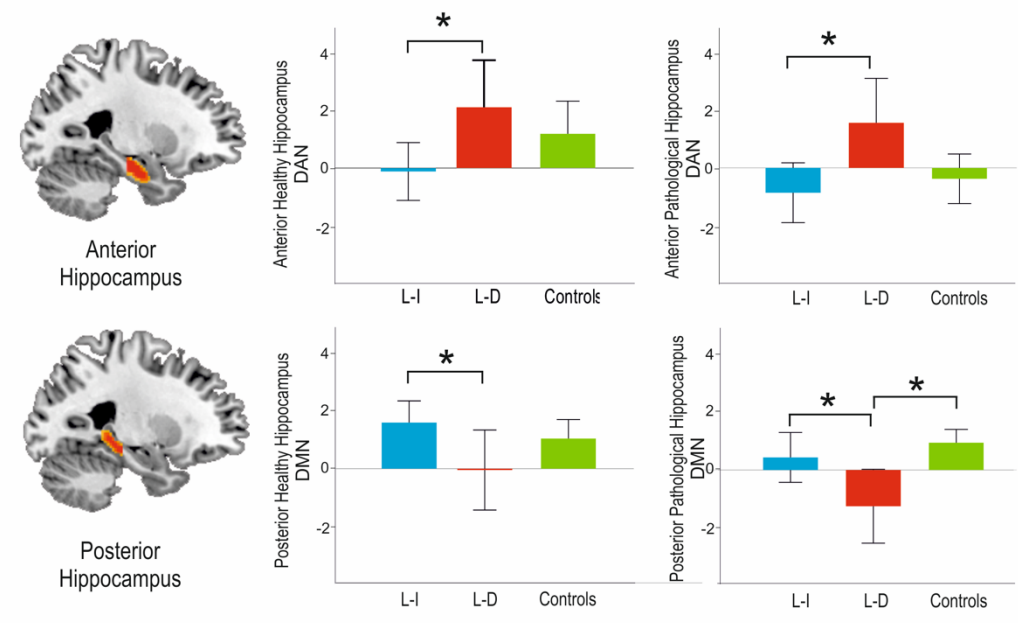


Figure 22. Main results of the post-hoc analysis among groups connectivity analyses with the DAN and the DMN along the longitudinal axis of the hippocampus. L-I= Learning Intact; L-D=Learning Decline. * Indicates statistical significance $p<0.05$.

The MANOVA was followed up with a linear discriminant analysis, which provided a multivariate model of how the connectivity measures differentiated the groups. As the discriminant analysis was carried out among three different groups, two different functions were obtained, as per differentiate each group. The functions tried to differentiate the three groups of participants considering a minimum weighted combination of the connectivity measures analyzed.

Using the connectivity of the hippocampus as a whole, the first function significantly discriminated the controls from the patients [Wilkin's lambda=0.17, $X^2=77.05$, $p<0.0001$], but the second function was non-significant [Wilkin's lambda=0.850, $X^2=7.06$, $p=0.216$], so the model was not capable of appropriately distinguishing the groups of patients, thus not identifying the learning-decline patients. On the other hand, for the anterior/posterior division results, the first discriminant function explained 63.3% of the variance, canonical $R^2=0.67$, and significantly discriminated the learning-decline patients from the other groups

[Wilkin's lambda=0.37, X^2=40.41, p=0.019]. Individual discriminant scores for this function correlated significantly with the connectivity between the posterior pathological hippocampus and the DMN (0.67), inversely with the connectivity between the anterior pathological hippocampus and the DAN (-0.63), and finally with the posterior contralateral hippocampus with the DMN (0.47). As such, these three variables demonstrated the most importance in terms of learning-decline group differentiation. The second discriminant function explained the 36.7% remaining variance, canonical R^2=0.570, but was not significant (Wilkin's lambda=0.851, X^2=7.03, p=0.218]). See Figure 23 for details.

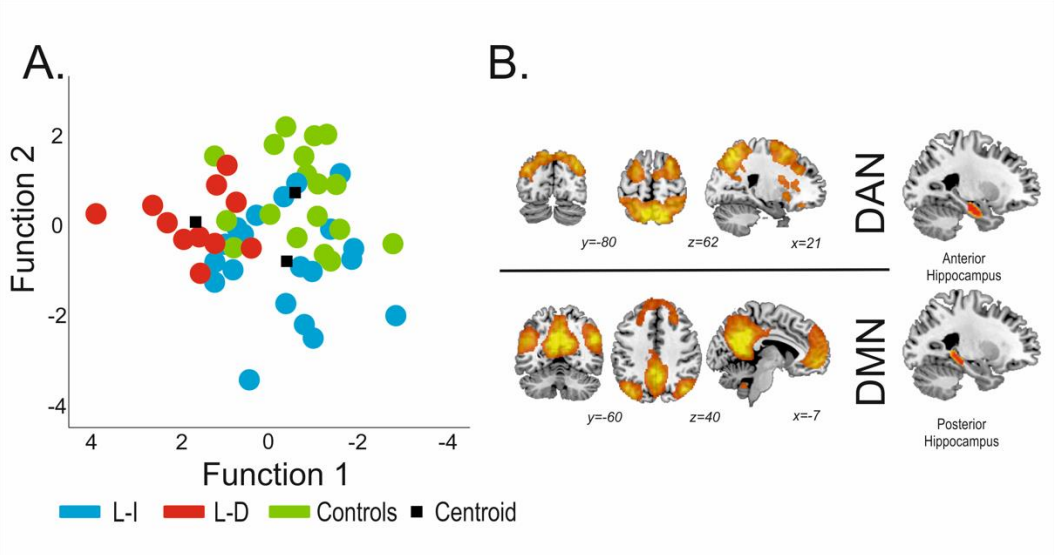


Figure 23. A. Canonical functions distribution of the discriminant analysis. Function 1 was found to significantly distinguish patients with decline from patients without decline and controls. **B.** The connectivity variables with higher load were both for the pathological hippocampus (posterior part indicates connectivity with the DMN, and the anterior portion indicates connectivity with the DAN).

Finally, a ROC curve was calculated for the optimal cut-off point to predict verbal learning decline from the three previous connectivity variables which showed significance in the discriminant analysis (posterior bilateral hippocampi to DMN and anterior to-be resected hippocampus to DAN). Also, for the connectivity of the whole hippocampus a ROC curve was calculated, using the bilateral DMN connectivity and the pathological hippocampus to DAN connectivity. The results for the hippocampus as a whole, presented an Area

under the Curve (AuC) was 0.63, with an optimal sensitivity of 0.45 and specificity of 0.8, whereas for the anterior/posterior connectivity presented an AuC of 0.84, with an optimal sensitivity of 0.73 and specificity of 0.95. See Figure 24.

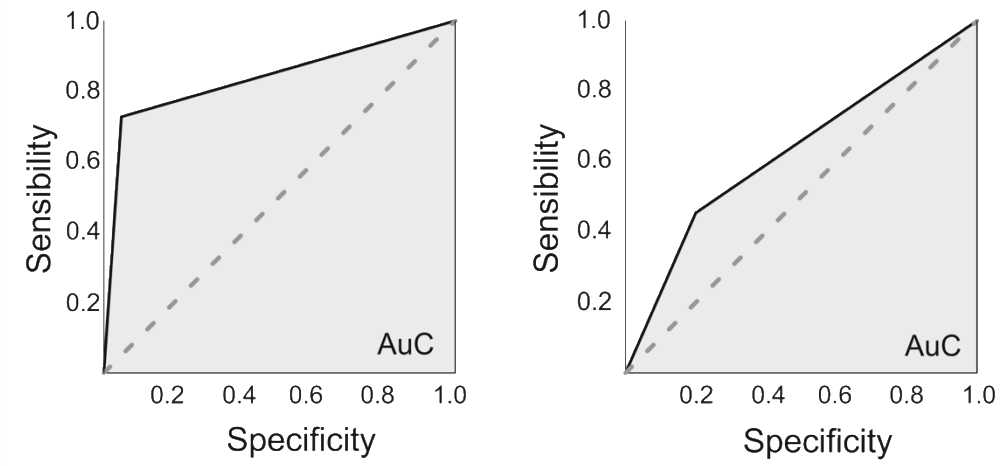


Figure 24. ROC curves. On the left, using the anterior/posterior division of the hippocampi (Connectivity measures for the Posterior bilateral hippocampi-DMN and the anterior to-be resected hippocampus-DAN). The area under the curve (AuC) was of 0.835, with an optimal sensitivity of 0.727 and specificity of 0.95.

On the right, using the whole hippocampus (also connectivity measures from the bilateral hippocampi-DMN and the to-be resected hippocampus-DAN). The AuC was 0.63, with an optimal sensitivity of 0.46 and specificity of 0.8.

4.3.4 Evaluation of the results

In this study, we identified functional connectivity signatures related to post-operative verbal learning decline in TLE patients. That is, different connectivity patterns with the RSN along the longitudinal axis of the hippocampus discriminated among patients with and without severe decline in verbal learning after surgery. In particular, we found a pre-operative increase of connectivity of the anterior to-be resected portion of the hippocampus with the DAN, together with a bilateral decrease of connectivity of its posterior portion with the DMN, that appropriately differentiated the group of patients with post-surgery learning-decline from learning-intact patients and controls. In addition, even though significant changes were found in connectivity among the anterior hippocampi and the EXE network, these changes did not sufficiently discriminate learning-decline patients according to our model.

Our study revealed two main levels of organization along the long hippocampal axis associated with the verbal learning decline after resection conferring protection against the cognitive impairment after surgery in TLE patients. First of all, according to our data, post-surgery learning-decline patients, learning-intact patients and controls differed in the pattern of the hippocampal formation connectivity with the RSN following an anterior-posterior gradient. Secondly, we found bilateral, up- and down-regulations of resting state connectivity, with significant differences in the learning-decline group versus the learning-intact and control groups. Finally, in our series, investigating the connectivity along the longitudinal axis proved to discriminate learning decline patients from learning intact patients and controls, as compared with the connectivity of the hippocampus as a whole, which was only capable of discriminating patients from controls.

In recent years, the modular paradigm, i.e., postulating specific brain areas as responsible of complex cognitive tasks, has been shifted towards the study of how neural networks

influence cognitive processing (Bressler et al., 2010). According to the network paradigm, cognitive functions arise from distributed brain areas comprising multiple distinct, interacting networks. Several large-scale brain networks have been described using resting state functional imaging. It is this connectivity of these RSN with other brain structures such as the hippocampal formation that has proven to be crucial for the maintenance of cognitive tasks (Chand et al., 2018; Smith et al., 2009).

Verbal learning decline has been a consistent finding after temporal lobe surgery (B. Bell et al., 2013). Two models were initially proposed as per why some patients showed verbal memory decline after surgery. Namely, these include functional adequacy of the to-be resected hippocampus as sustaining cognitive function, which is thereby lost upon resection, or instead the functional reserve of the contralateral hippocampus (Chelune, 1995). However, more recent work switched the attention from the hippocampal model to the connectivity of widespread neuronal networks in order to explain complex cognitive changes that imply different functions (Bressler et al., 2010). In this regard, aligned task-based functional MRI studies using a combination of language and encoding paradigms showed both hippocampal and extrahippocampal activation differences in identifying patients at risk of decline. Thus, the authors postulated that it was the functional adequacy of the network elucidated in the task what was truly relevant for verbal memory decline (Sidhu et al., 2015). Taken together, these results, along with evidence on targeting network dysfunction for neurological symptoms (Boes et al., 2015), paved the way for analysis beyond the lesional level, exploring the cognitive deficits at a network level. Our main hypothesis builds up from this perspective, exploring the role of the disconnection of the hippocampus with the DMN, DAN and EXE, crucial in verbal learning (Chand et al., 2018; Persson et al., 2018).

Our main findings are in line with this network model. Analyzing resting-state connectivity of the hippocampus with the DMN, DAN and EXE, bilaterally and along its longitudinal axis, significantly discriminated patients at risk of verbal memory decline; a bilateral decrease of the posterior portion of the hippocampi, together with an increased connectivity of the to-be resected hippocampal anterior portion with the DAN was significantly related to verbal learning decline after surgery. Noteworthy, hippocampal connectivity with the RSN followed a functional gradient, that is, the posterior hippocampus with the DMN and the anterior hippocampus with the DAN. Thus, by segregating the connectivity pattern along the axis of the hippocampus, we found significant differences related to the connectivity of the anterior-posterior system (Grady, 2019; Ranganath et al., 2012).

Previous studies evaluating resting state connectivity also reported differences among patients with and without decline (G. E. Doucet et al., 2015; Mccormick et al., 2013; Roger et al., 2020). In this sense, the definition of different cognitive networks at rest provides an easy and reproducible framework to assess brain functioning. Network studies at rest in TLE patients have shown widespread alterations and correlations to different neuropsychological tasks (G. E. Doucet et al., 2015; Voets et al., 2014; Zheng et al., 2012). Using resting-state connectivity, previous studies showed how the connectivity of the hippocampus and the posterior cingulate, a key hub in the DMN, differed among patients. Relating to their different verbal memory scores, lower strengths of connectivity between the DMN and the hippocampi correlated with poorer performances (Holmes et al., 2014). Furthermore, changes in DMN-hippocampal connectivity may relate with memory loss after surgery (Mccormick et al., 2013). Specifically, evaluating the connectivity of the posterior cingulate within the DMN to the hippocampus elucidated how patients with episodic memory loss after surgery showed stronger connectivity with the pathological hippocampus, whereas intact patients showed stronger connectivity with the contralateral hippocampus.

In addition, using graph theory measures of areas relevant to cognitive functions and to ictal pathology, Doucet et al. (G. E. Doucet et al., 2015) found that neurocognitive deficits after surgery were related mainly to the contralateral hippocampus and widespread bilateral regions, again emphasizing the importance of extratemporal of networks in cognitive functioning.

Nonetheless, the variety of approaches to analyze resting state data, together with the large number of neurocognitive tests reported, limits study comparison. In relation to previously reported studies, we did also find a decrease in contralateral posterior hippocampus-DMN connectivity for the learning-decline patients, but, on the contrary, this group also displayed decreased connectivity between the pathological posterior hippocampus and the DMN. In our approach, we also found that the connectivity of the anterior hippocampus and the DAN was a major discriminant of learning-decline patients. This connectivity has been reported as crucial for encoding processes (Fritch et al., 2020; Kim, 2015). We hypothesized that an increased connectivity of the anterior pathological hippocampus to the DAN could relate to adaptive changes, relating to the functional adequacy of the to-be resected hippocampus. This pattern of the to-be resected hippocampus, having decreased posterior connectivity to the DMN bilaterally, but increased anterior connectivity to the DAN, could related to a higher vulnerability compared to the learning-intact, who showed the inverse pattern (see Figure 27).

Our series consists of patients with different etiologies, with a majority of patients having HS. TLE is an heterogeneous disease, even in patients with HS, were heterogeneity in impairment of cognitive domains is found (Prada Jardim et al., 2017). Individual differences are common, likely related to network plasticity (Bettus et al., 2009) against different etiologies and ages of presentation. In our results, different network connectivity of anterior and posterior hippocampus in patients and controls was found, likely related to the different

network readjustments in our group of TLE patients, as TLE disrupts large scale cognitive networks (Cataldi et al., 2013). Analyzing the differences along the longitudinal axis of the hippocampus could help identify patients at risk of cognitive decline after surgery. In our series, the patients who suffered a verbal learning decline relied on increased connectivity of the anterior pathological hippocampus to the DAN, with decreased connectivity of the posterior hippocampus with the DMN. After resecting the anterior portion of the hippocampus, it is assumed that this connectivity would be disrupted, and therefore the patients suffered a verbal learning impairment.

Our study has several drawbacks. First, there was a small number of patients included. Second, we did not consider the extent of resection of the mesial and lateral temporal lobe, which is also associated with memory impairment after surgery (Bruce P. Hermann et al., 1996). Also, most of our sample consists of patients with HS, which could mean that our findings are specific of this condition. Finally, we did not find significant differences between learning-intact and controls, as the discriminant function was not able to differentiate between these groups. Although research supports that intact cognitive patients with TLE may be comparable to healthy population (Reyes et al., 2019), further studies with higher number of participants could be necessary in order to better delineate the learning-intact patients.

On the other hand, applying network analysis along the functional gradient of the hippocampal formation, we report pre-surgical differences among patients with verbal learning decline probably related to individual network plasticity. This finding supports the assumption that RSN – hippocampal connectivity is relevant for cognition, and measuring its integrity could help in the pre-surgical assessment of cognitive risks. However, several other factors should be considered, furthermost the extent of resection, pre-operative memory testing, and verbal lateralization. Nonetheless, the pre-operative connectivity of the mesial

temporal area could help predict the risks of verbal learning decline after surgery for TLE. A multivariate prediction system would likely be the best approach in predicting memory deficits after surgery.

These findings support the hypothesis of an anterior-posterior functional division of the hippocampal formation and the cognitive networks. Differences in the pattern of functional connectivity of the DAN and DMN along the longitudinal axis of the hippocampus may have implications on post-operative cognitive deficits and could help identify individually which patients are more at risk of cognitive impairment. Verbal learning impairment remains an important side effect of epilepsy surgery. As a result, personalized counseling based on resting state network connectivity could help in decision making.

4.4 *STUDY 4. De novo depression following temporal lobe epilepsy surgery*

TLE and mood disorders share a common underlying network of structures responsible for their pathogenesis. For patients with drug resistant epilepsy, surgical removal of the mesial temporal lobe can improve and even cure seizures; however, this may lead to mood disorders in patients with no previous psychiatric condition.

In this study, our objective was to examine whether the preoperative connectivity between two pivotal structures within this interconnected network, namely the Hippocampus and the NAcc of the hemisphere slated for surgery, exhibited an identifiable pattern among patients who subsequently developed a mood disorder following the procedure.

With this purpose, we included 27 patients with TLE and 18 controls. All participants underwent an 3T structural MRI; for the patients, the same structural sequence was performed before and after a temporal lobe resection for intractable epilepsy. Besides, all patients and controls underwent a gambling reward task during functional MRI. After surgery, patients were followed-up; those who developed depression the first year after surgery without a prior history of mood disorders were classified as DnD; those who did not were classified as nD.

For all participants, a first level analysis between gains and losses was performed, and we computed connectivity maps between the functional NAcc and the whole brain, extracting r-correlation scores that were then converted into z-scores. In parallel, for patients undergoing surgery, a mask of the resected Hippocampus was created manually with the post-op structural sequence. This mask was then applied to the preoperative structural MRI. Finally, we compared the strength of connectivity of this area Hippocampus to be resected and the functional NAcc, by using a two-tailed Crawford's modified T-test. This test is

specially design to individually compare patients with as sample of control participants, and provides and individual T-value for each patient against the set of controls. Then, we compared the individual T-values of patients with DnD with those with nD.

Within the initial year post-surgery, post-surgical de DnD emerged in 7 out of 27 patients. The majority of patients (88.8%, n=24) exhibited a significant reduction in NAcc – Hippocampus connectivity compared to the control group. We scrutinized the extent of this connectivity reduction (measured by the absolute individual T-value for each patient relative to controls) for the two cohorts, namely DnD and nD: the disparities in individual connectivity were statistically significant (median -2.1 for DnD vs. median -2.02 for nD, Mann-Whitney U=13, p=0.006), highlighting notably lower connectivity values in the DnD group compared to the nD group. To ascertain the most suitable threshold for evaluating the risk of post-surgical depression, we performed a ROC curve analysis. The findings revealed an AuC of 0.87, with the optimal cutoff point at a Crawford-T value of -2.08. This threshold demonstrated a sensitivity of 0.83 and a specificity of 0.76.

Connectivity patterns within the reward network could potentially serve as a biomarker for predicting the onset of de novo mood disorders in TLE patients undergoing surgery. This insight could contribute to identify and offer guidance to individuals before undergoing the surgical procedure.

4.4.1 Background

In order to better understand the impact of surgery on mood disorders, several studies have investigated whether mood symptoms improve or worsen after surgery (Cleary et al., 2013). Patients' prior psychiatric history, specifically depression, anxiety, and chronic pain, have been described as the foremost predictors of post-surgical mood decline. Other factors such as age at surgery, neuropsychological profile, and marital status have also been identified as contributing factors (Doherty et al., 2021; Wrench et al., 2011a). However, it is noteworthy that *de novo* depression – the onset of depression in patients with no prior history of mood disorders – has been consistently observed in patients undergoing temporal lobe surgery. The percentage of patients who develop DnD can vary, with reported rates reaching up to 38% among those undergoing surgery (Hue et al., 2022). DnD typically occurs within the first year after surgery, showing higher prevalence following the resection of temporal lobe structures, as in TLE surgery, in comparison to the resection of other brain structures for treating different types of drug-resistant epilepsies. Interestingly, DnD does not seem to be related to seizure relapse (Wrench et al., 2011b).

Mechanistically, the occurrence of DnD after temporal lobe surgery has been suggested to be related to limbic disfunction (Altshuler et al., 1999). Epidemiological studies have shown a high frequency of depression among drug-resistant TLE patients, attributed in part to the shared underlying pathological network of structures. Research has evidenced links between mood disorders and blunted responses to reward (Huys et al., 2013). In this context, studies investigating the neurobiological basis of reward processing have identified a network of structures that includes the NAcc amygdala, hippocampus and anterior cingulate cortex (Camara, Rodriguez-Fornells, Ye, et al., 2009), all of which are involved in the network underlying TLE (Spencer, 2002). Aligned task-based fMRI studies have revealed that patients with mood disorders exhibit decreased activity in limbic and subcortical structures, including the NAcc and Hippocampus (W. N. Zhang et al., 2013). In fact, a decrease

in NAcc functional connectivity with the anterior cingulate cortex during a reward task has been associated with the severity of depressive symptoms in a cohort of patients (Rupprechter et al., 2020). Additionally, the Hippocampus has been implicated in mood disorders as a key structure in the reward network (Axmacher et al., 2010). Specifically, the Hippocampus is involved in instrumental reward based learning (Vilà-Balló et al., 2017), and its plasticity properties appear to be crucial in depression (Tartt et al., 2022). Finally, recent evidence demonstrated these blunted responses to reward in a cohort of TLE patients when compared against controls (Vilà-Balló et al., 2022).

Considering that TLE surgery commonly involves the unilateral removal of the Hippocampus (Wiebe et al., 2001), it prompts us to consider the potential consequences of this disconnection on the reward network. This disconnection may give rise to symptoms resembling DnD due to network dysfunction (Boes et al., 2015). In this context, the emergence of *de novo* mood disorders, temporally linked to the surgical procedure, in patients with no prior psychiatric history offers a valuable framework for examining the hypothesis that disruptions in brain network connectivity may underlie symptoms in previously well-adjusted patients.

In this study, our primary objective was to explore whether the strength of functional connectivity between the NAcc and the Hippocampus, which was later resected, could serve as a predictive biomarker for mood deterioration in patients with TLE who developed DnD following mesial temporal resections. To achieve this aim, we employed fMRI during a reward-related task prior to the surgical procedure to identify the functional NAcc in a cohort of patients scheduled for anterior temporal resections due to TLE. Subsequently, we conducted post-surgery scans to delineate the resected hippocampal tissue. As a result, we were able to gauge the Functional NAcc connectivity to the precise, designated individual region in the Hippocampus slated for resection before the surgical procedure. To delve

deeper into individual patterns, we scrutinized the connectivity of each patient in comparison to the control group, thereby avoiding group-level averaging which can miss relevant effects in patients with epilepsy (Lee et al., 2022; Lo et al., 2015).

Our hypothesis centered around the notion that the Hippocampus-NAcc connectivity before surgery would act as a biomarker for mood decline after the surgery. Consequently, we focused our investigation exclusively on the connection slated for resection, without assessing contralateral connectivity or other structures that are also part of the reward network.

On an individual basis, we compared the pre-surgical functional connectivity with a control group to examine single-subject connectivity dysfunction. We hypothesized that patients who developed DnD post-surgery would exhibit distinct pre-surgical connectivity patterns between the functional NAcc and the Hippocampus scheduled for resection.

4.4.2 Experimental design

4.4.2.1 Participants

We performed a prospective observational study of a cohort of patients undergoing epilepsy surgery in Bellvitge hospital surgical program. From 2009 to 2014, 33 patients with TLE drug-resistant epilepsy were included in our epilepsy surgery program and underwent a complete work-up with Video-EEG, MRI and neuropsychological evaluation. Patients were diagnosed and follow-up by expert epileptologists and were specifically evaluated for psychiatric history and symptoms. When psychiatric pathology was present, patients were also evaluated and followed-up by a psychiatry specialist. We also included 22 healthy individuals matched for handedness, age, gender, years of education and without any history of psychiatric disorders.

This study involving human participants adhered to the ethical principles outlined in the Declaration of Helsinki. The research protocol has been approved by the ethic committee of Hospital de Bellvitge and written informed consent has been obtained from all participants involved in the study. Participants were assured of confidentiality, voluntary participation, and the right to withdraw without prejudice.

After obtaining the patients' agreement for surgery and participating in the study, we conducted a pre-operative MRI neuroimaging protocol, encompassing an anatomical sequence, a reward-based functional task, and resting-state sequences. After surgery, only the anatomical sequence was repeated.

Of the initial 33 patients, 31 underwent surgery, and 27 underwent the complete protocol (4 patients did not complete the post-operative MRI sequences). All patients underwent an anterior temporal resection by the same surgeon, and anatomopathological analysis was carried out by a single pathologist at our center.

Finally, 27 patients (14 women) were included in the study; median age at surgery was 41 y-o (range 23-66 y-o), and 13 had right TLE. Prior to surgery all patients were evaluated by epileptologists specifically questioning for mood disorders. Additionally, 13 patients with mood complaints underwent structured interviews with psychiatrists, who made diagnosis based on DSM-IV-criteria (C. C. Bell, 1994). Out of the 13 patients who underwent psychiatric evaluation, 6 patients were diagnosed with a major mood disorder (5 with major depressive disorder, and one with bipolar disorder), while the remaining 8 patients did not reach diagnostic criteria.

After surgery, median time of follow-up was 5 years (range 3-7 years), with all patients regularly undergoing prospective follow-up in our epilepsy clinic. The first visit after epilepsy surgery took place one month after the procedure, with subsequent regular evaluations every three to six months. At the end of the follow-up period, 51.9% of the patients (n=14) were classified as completely seizure free. Within the first year after surgery, 7 patients with no prior history of psychiatric illness (26%) developed DnD, which was confirmed by a psychiatrist according to DSM-IV. The median time to onset of symptoms was 4 months (range 1 to 8 months), and none of these patients had a history of mental disorders. We classified these patients as DnD and compared them to the remainder of the cohort (referred to as No *de novo* Depression, or nD).

All participants underwent a pre- and post-operative whole-brain structural MRI scan using a 3.0 Tesla Siemens Trio MRI. A 32-channel phased-array head coil system was used to acquire high-resolution T1-weighted images (slice thickness=1mm; no gap; number of slices=240; TR=2300ms, TE=3ms, matrix=256x256; FOV=244mm; voxel size 1x1x1mm). Resting state fMRI data were collected using a single-shot T2*-weighted gradient-echo EPI sequence (slice thickness = 4 mm; no gap; number of slices = 32, interleaved order; TR =

2000 ms; TE = 29 ms; flip angle= 80°; matrix = 80 Å~ 80; voxel size = 3 Å~ 3 Å~ 4 mm3, 110 volumes).

In this study, prior to surgery, patients performed a reward-related fMRI task and resting-state scanning. Specifically, we used fMRI during a gambling task in a cohort of TLE patients and controls. This task was designed to elicit reliable reward-related brain activity, allowing us to localize the functional NAcc, a core area in the reward circuitry. After the surgery, patients underwent a structural MRI sequence in order to delimit the resected hippocampal tissue. In addition, by using pre-surgical resting-state fMRI data, we assessed the functional connectivity between the to-be resected side Nacc and the whole brain in controls and patients (see methods below). Then, we conducted individual-based comparison of functional connectivity between each patient and a group of healthy controls to identify those voxels with abnormal connection dysfunction. Finally, we compared the strength of connectivity between the NAcc and the-to-be resected Hippocampus in the altered voxels between patients who developed DnD and those who did not.

It is important to note that in order to account for differences in the side of the epileptic focus, images of patients with right TLE, as well as an age-matched control group, were flipped. This procedure ensured that all affected epileptic foci were located on the left hemisphere. At this point, all subsequent analyses referred to the ipsilateral hemisphere in relation to the epilepsy focus.

4.4.2.2 Functional MRI task:

In order to accurately identify the functional NAcc in the hemisphere targeted for surgical intervention, we used a modified version to the monetary gambling task developed by Camara et al., (Camara, Rodriguez-Fornells, Ye, et al., 2009) (adapted from Gehring and Willoughby (Gehring et al., 2002)). This task has been widely used to assess the processing of rewards also in TLE (see also Vila-Batllo et al., (Vilà-Balló et al., 2022)). The task began

with a fixation point, followed by the display of two numbers in one of two possible combinations: [25 5] or [5 25]. Participants were required to bet on one of the two numbers, by selecting it with the corresponding left or right index finger. One second after the choice, one of the numbers turned green and the other turned red. If the number selected by the participant turned red, they lost the corresponding amount of money, while a green number indicated a gain in Euro Cents. In addition to the standard trials described above, unexpected large gain or loss trial occurred in 10% of the trials. In these trials, regardless of the number chosen (either 5 or 25) the feedback displayed a value of 125.

Participants received an initial sum of 10 € and were motivated to win as much money as possible. The experiment consisted of 3 runs, each containing 383 trials. After each run, participants were informed about the accumulated amount of money. At the end of the experiment, participants were paid the final amount they had earned. The experiment was designed as an event-related paradigm (40% Gain, 40% Loss, 10% Boost Gain, 10% Boost Loss). (See Figure 25A for design details).

4.4.2.3 Functional MRI pre-processing:

Task-based functional images were preprocessed using standard protocols implemented in the Statistical Parameter Mapping software (SPM12, http://www.fil.ion.ucl.ac.uk/spm). Initially, slice-timing correction was performed to account for the time difference between brain slice acquisitions. Subsequently, an affine rigid-body transformation with the first brain volume as a reference was performed to correct for head motion. Then, realigned functional data was averaged and the mean functional image was normalized to the standard MNI template (ICBM 152, Montreal Neurological Institute) provided by SPM12. The normalization method included an initial 12-parameter affine transformation, with iterative non-linear normalization involving discrete cosine basis functions implemented in SPM12 (J Ashburner et al., 1999). The resulting normalization parameters, derived for the mean

image, were then applied to the whole functional set. Finally, functional data was spatially smoothed using an 8 mm full-width half-maximum (FWHM) isotropic Gaussian Kernel to minimize effects of inter-subject anatomical differences.

Statistical analysis was carried out using a General Lineal Model based on a least-square estimation. Experimental conditions were modelled using a box-car regressor waveform and convolved using a canonical hemodynamic response function (HRF) (Friston et al., 1998). To account for movement-related noise, the six rigid-body motion parameters were included as nuisance regressors in the design matrix. In addition, we also included the fixation and the response conditions as regressor of non-interest. Data were high-pass filtered and serial autocorrelations were estimated using an autoregressive model. Regressors of interest included the experimental conditions of Gain5, Gain25, Gain125, Loss5, Loss25 and Loss125. After model estimation, the main effect of each condition was calculated, and main contrast were assessed. Gain (Gain5 + Gain 25 + Gain 125) vs. Loss (Loss5 + Loss25 + Loss 125).

For the second level analysis, first-level contrasts of patients and controls were entered into a one-sample *t* test. Effects were considered significant at a whole-brain level if they exceeded a voxel-wise threshold of $p<0.001$ and a minimum cluster extent of 20 contiguous voxels. Multiple comparisons were corrected cluster-level FWE correction, with a significance threshold of $p<0.05$. (See Figure 25B).

4.4.2.4 Region of interest: NAcc localization & Resection delimitation

The NAcc was defined based on the activation observed in the Gain vs Loss contrast in the functional gambling task. Specifically, this cluster represents the main group effect located in the left NAcc, corresponding to the ipsilateral side in relation to the epileptic focus, in a second level analysis that involved both the control group and patients.

To identify which regions were removed by surgery, we manually delimitated the resected tissue in native space of each patient. This involved using the postoperative T1 weighted image and the MRIcron software package (http://www.cabiatl.com/mricro/mricron/index.html) to delineate the extent of the surgical intervention. The resulting masks were depicted by an expert neurologist and encompassed the temporal resections. We next, normalized the resected mask to MNI space using unified segmentation and cost function masking (John Ashburner et al., 2005; Ripollés et al., 2012). (See Figure 25C).

4.4.2.5 Brain Connectivity analysis

We used standard protocols implemented in the CONN-fMRI Functional Connectivity toolbox (www.nitrc.org/projects/conn). After slice-timing correction, functional images were realigned to the first volume using rigid body spatial transformations to correct for motion artifacts. Then, structural images were co-registered to functional images and images were spatially normalized into MNI space and resliced to a 2-mm isotropic resolution. In addition, normalized images were smoothed using a 8-mm full-width at half maximum (FWHM) isotropic Gaussian kernel. Finally, to minimize potential confounding effects, we regressed out white matter, and cerebrospinal fluid physiological noise sources together with the realignment parameters (Behzadi et al., 2007). Following this step, images were band-pass filtered (0.008 and 0.09 Hz) to attenuate low frequency drift and high-frequency noise artifacts.

For the first-level analyses, we computed functional connectivity maps between the functional NAcc and the whole brain for both patient and control groups. This involved extracting the mean time-BOLD signal from the seed region and using it as a regressor to calculate voxel-based correlations. These r-correlation scores were then converted to z-scores using Fisher transformation, enabling to estimate the strength of functional connectivity at whole brain level.

To identify abnormal functional connectivity in individual patients, we conducted a voxel-wise analysis in the to-be-resected area, comparing the strength of connectivity with the NAcc. Specifically, we employed a two-tailed Crawford's modified T-test, which is designed to compare a patient to a small sample of control participants (J. Crawford et al., 1998; Tuomiranta et al., 2014).

We considered connectivity to be altered when the differences were statistically significant at $p<0.005$. Next, we averaged the T-values of all the significant voxels within the resection masks for each participant. This average represented the magnitude of altered functional connectivity between the to-be-resected Hippocampus and the ipsilateral NAcc and provided us with individual T-values of each patient against the group of controls. Finally, we compared these individual T-values between DnD and nD groups.

We conducted statistical analyses using SPSS v.26 (SPSS Inc, Chicago, USA). To compare the sociodemographic and clinical data, as well as the altered functional connectivity magnitude between the DnD and nD patient groups, we used T-student and Mann-Whitney U tests for quantitative variables, and the Xi-square or Fisher exact F for qualitative variable comparison. Finally, a ROC curve was calculated in order to check the best cut-off points to individually classify patients with DnD and nD depending on the individual T-connectivity value.

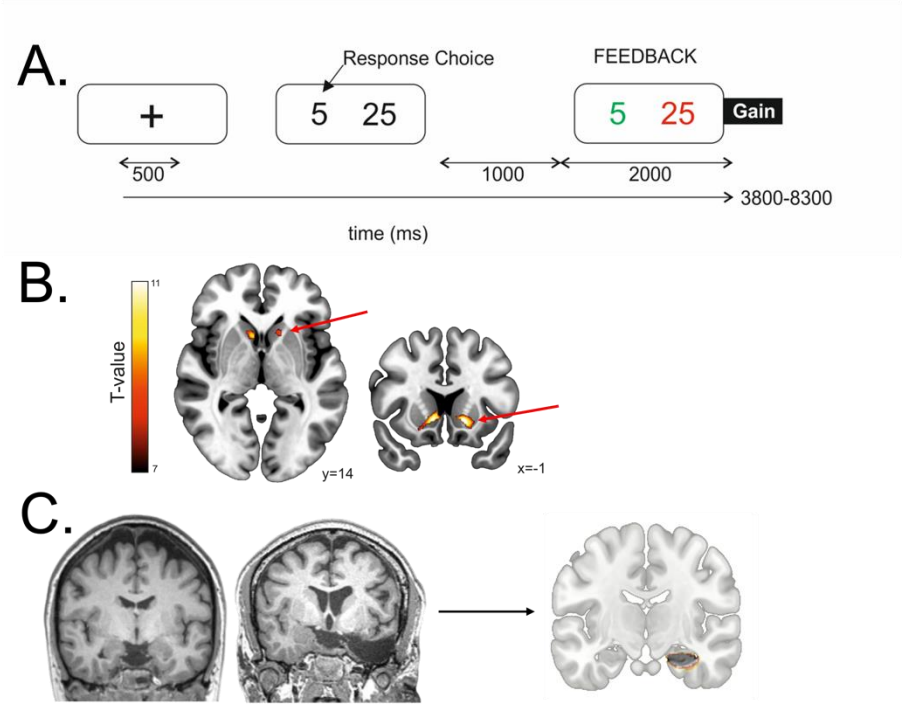


Figure 25. On top (A), Reward task diagram of the functional MRI.

Bottom left (B), group level analysis of activation for the gain vs loss contrasts, including patients and controls. First-level contrasts of patients and controls were entered into a one-sample *t* test. Effects were considered significant at a whole-brain level if they exceeded a voxel-wise threshold of $p<0.001$ and a minimum cluster extent of 20 contiguous voxels. Multiple comparisons were corrected cluster-level family wise error (FWE) correction, with a significance threshold of $p<0.05$. Red arrow points to final NAcc ROI selected to extract connectivity values. All patients with right sided surgery were reversed.

Bottom left (C), patients were scanned prior and after surgery; then, individual masks of the resected hippocampus were extracted for each patient. We then calculated the connectivity between the resected hippocampus and the ipsilateral NAcc

4.4.3 Results

Within the first year after surgery, 7 out of 27 patients developed post-surgical DnD. Baseline characteristics of these patients, as well as the rest of the sample are detailed in Figure 26. There were no significant differences in the age of seizure onset (U=40.50, p=0.104), the side of the surgery (X^2=4.34, p=0.077), or seizure freedom after surgery (X^2=4.34, p=0.077), between the DnD and nD patient groups.

	De novo Depression (DnD)	no Depression (nD)	p
Age at surgery. Mean y-o (SD)	45 (11)	42 (12)	0.578
Women n (percentage)	5 (71.4)	9 (45)	0.385
Left TLE n (percentage)	6 (85)	8 (40)	0.07
Age of onset Mean y-o (SD)	8 (8.1)	16 (11.6)	0.07
Seizure freedom n (percentage)	6 (85.7)	8 (40)	0.07

Figure 26. Demographic and clinical characteristics of the TLE patients. TLE (temporal Lobe epilepsy); y-o (years-old); SD (standard deviation)

For the Gain vs Loss contrast, both the control group and patients engaged the reward network including bilaterally the NAcc (To be resected side: peak coordinates: x=-8, y=15, Z=-5 mm; T=5.56, k=30; $p < 0.05$; Non resected side: peak coordinates: x=9.5 y=10 Z=-1.5 mm; T=4.71, k=38; $p < 0.05$; FWE at whole-brain level; 20 voxels spatial extent).

Most patients (88.8%, n=24) showed a significant decrease of NAcc – Hippocampus connectivity when compared to controls. In terms of groups, both the DnD patients (85%, n=6) and the nD patients (85%, n=17) showed a significant decrease in connectivity compared with controls. Then, we compared the magnitude of the decrease of connectivity (absolute individual T-value obtained for each patient against controls) for the two groups,

DnD and nD: the differences in individual connectivity were significant (median -2.1 for DnD vs median -2.02 nD, Mann-Whitney U=13, p=0.006; see Figure 27) showing the DnD group significant lower connectivity values than the nD. Additionally, we compared both groups excluding the patients with mood disorders already diagnosed before surgery (n=5); the DnD patients still exhibited significant decreased connectivity when compared to the nD patients (Mann-Whitney U=10, p=0.009)

To determine the optimal cut-off point for assessing the risk of post-surgical depression, we used a ROC curve analysis. The results indicated an AuC of 0.87, with an optimal cut off point at Crawford-T value of -2.08. This cut of point displayed a sensitivity of 0.83 and a specificity of 0.76. See Figure 27.

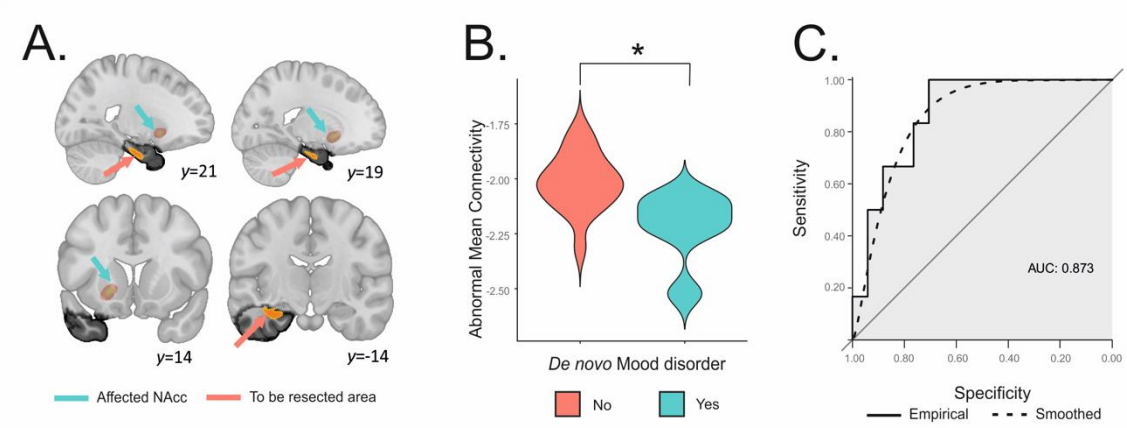


Figure 27. A. For each patient, the connectivity between the functional NAcc (blue arrow) and the to-be-resected Hippocampus (red arrow) was calculated, and compared with the groups of controls, in order to get individual T values.

B. Patients with DnD showed a significant decreased individual pre-surgical connectivity compared with nD.

C. ROC curve with the individual connectivity values, with an AuC of 0.87, and an optimal sensitivity of 0.83 and specificity of 0.76

4.4.4 Evaluation of the results

In this report we investigated the pre-surgical functional connectivity within the reward network involving two key structures, the NAcc and the Hippocampus, and the potential relationship between this connectivity and the development of post-surgical *de novo* mood disorders following Hippocampus resection in patients with drug resistant TLE. Our findings revealed that patients with lower connectivity values between the to-be resected Hippocampus and the NAcc were more likely to experience DnD following epilepsy surgery. These results suggest that functional connectivity patterns between these regions may play a role in predicting the risk of post-surgical mood disorders in patients undergoing Hippocampus resection.

DnD has been extensively reported in series of patients after undergoing temporal lobe epilepsy surgery, with limited research focusing on *de novo* psychiatric disorders rather than changes in preexisting psychiatric conditions (Cleary et al., 2013; Wrench et al., 2009, 2011b). In patients with TLE who undergo surgery, a complex interplay of personal, social, and biological factors converge to contribute to the etiopathogenesis of mood disorders (Ramos-Perdigués et al., 2018; Wrench et al., 2011b). In addition to known contributing psycho-social factors (Wrench et al., 2009), there is evidence suggesting that the resection in the mesial temporal lobe may play a crucial role in the appearance of mood disorders following epilepsy surgery (Wrench et al., 2011b). For instance, higher incidence of DnD has been observed after mesial temporal lobe compared to non-mesial resections (Wrench et al., 2011a). Despite early postulations implicating limbic structure disruption in the development of depression symptoms (Altshuler et al., 1999; Ring et al., 1998), no previous studies have been focused on interrogating the pre-surgical connectivity of the reward network. This gap in literature motivated our investigation of the functional connectivity between the NAcc and the to-be-resected Hippocampus in relation to the risk of DnD.

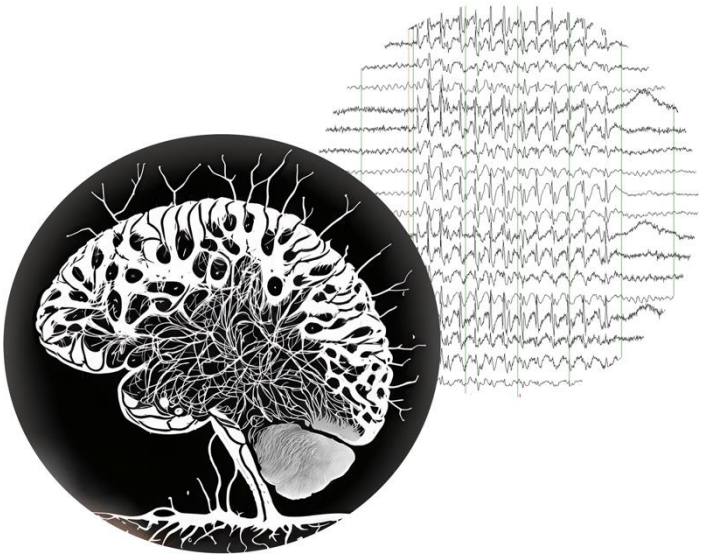
Our study focused on exploring potential biomarkers within the connectivity of the reward network, specifically the Hippocampus and the NAcc. We identified that a reduction in connectivity between these structures could be a risk factor for post-surgical depression in patients without prior history of psychiatric disorders. The underlying connectivity pattern of depression is not yet fully understood, since the dysfunction of several neuroanatomical structures has been associated to the manifestation of clinical symptoms (Ding et al., 2022). Previous research has consistently shown a decrease in the NAcc connectivity (Rupprechter et al., 2020) and Hippocampus atrophy (Tartt et al., 2022) in depression. As mentioned previously, the appearance of symptoms time-locked to the resection suggest a degree of lesional origin of those symptoms. Our findings suggest that the resection of already impaired connectivity could contribute to the appearance of depression, shedding light on the neurobiological basis of this disease. However, both the NAcc and the Hippocampus are part of a wider set of bilateral structures within the reward network, such as the amygdala and the anterior cingulate cortex, which were not investigate in the current study (Camara, Rodriguez-Fornells, Ye, et al., 2009). The involvement or preservation of these structures, as well as contralateral compensation of NAcc and Hippocampus, could also account for differences among patients, explaining why some patients with impaired NAcc-Hippocampus connectivity prior to surgery did not develop clinical depression. Preservation of these structures and contralateral compensation may play a role in the appearance of de novo mood disorders.

The relationship between socio-biographical events, changes in connectivity, and their causality or correlation remains unclear. (Disner et al., 2011). Furthermore, the underlying biological mechanism of mood disorders is not fully understood, but there is evidence of connectivity readjustments thein the limbic-reward system, as described previously (Duman et al., 2019; W. N. Zhang et al., 2013). Based on our findings, the pre-surgical connectivity

between the NAcc-Hippocampus could be a contributing factor among other social and personal variables that may trigger the appearance of DnD in TLE patients after surgery and might aid in pre-surgical counselling as well as more tailored resections in selected patients.

Finally, while our study has significant findings, it is limited by a small sample size. Therefore, further studies are warranted to replicate the finding that NAcc-Hippocampus connectivity serves as a biomarker for the development of depression in larger patient cohorts. Additionally, we did not account for other potential social factors that could contribute to mood disorders, such as marital status or employment, and not having a direct evaluation of connectivity after the surgery, and to compare it with pre-levels is also an additional limitation. However, it is worth noting that our sample was thoroughly selected and evaluated by psychiatrists to ensure accurate diagnoses of depression.

DISCUSSION



5 DISCUSSION

5.1 Summary of the results

In this doctoral thesis, we present four experiments aimed at investigating sources of individual differences in clinical manifestation of TLE patients after surgery. More specifically, we study the role of various biomarkers in predicting the prognosis of TLE surgery in three different clinical domains: seizure freedom, cognitive decline, and mood disorders. As evidenced by years of research, the understanding of TLE has evolved from viewing it solely as a localization-related disease focused on structures within the affected temporal lobe. Instead, it is now recognized as a neuronal network disease where the interaction and connectivity of a collection of distant and bilaterally distributed structures play a crucial role in its pathogenesis, comorbidities, and response to treatment.

Our research involves structures of different networks associated with TLE beyond the affected temporal lobe. We particularly focused on connectivity measures, encompassing both microstructural and functional aspects. Our multidisciplinary approach examines how assessing these biomarkers before surgery, from a network perspective, could significantly contribute to predicting the risk at individual-level associated with different outcomes, including seizure recurrences, cognitive deficits, and mood disturbances (See Figure 28).

5.1.1 Structural connectivity of the hippocampus and seizure outcome after surgery

In *Study 1*, we examined whether the microstructural architecture of the contralateral hippocampal subfields, relative to the resection site, influenced seizure outcomes in patients with confirmed TLE-HS on pathology analysis. Prior to surgery, non-SF patients exhibited statistically significant MD increases in CA1, molecular layer, and dentate gyrus of the contralateral hippocampus. Using a Cox-Regression model, MD values of CA1 and molecular

layer independently predicted seizure recurrence. Patients with lower MD values in contralateral molecular layer and CA1 remained seizure-free during long-term post-operative follow-up, showing a significant difference from non SF patients (Figure 11).

The subfields involved in these significant differences between groups (namely CA1, dentate gyrus, molecular layer) are known to be crucial to epileptogenesis and suggest an extended epileptogenic network, supported by prior research (Bernhardt et al., 2016; Sierra et al., 2015). Although bilateral changes are common in unilateral TLE-HS, not all patients with bilateral alterations experience persistent seizures; we proposed critical damage translates to prognosis, with specific molecular layer and CA1 mean diffusivity values predicting long-term seizure freedom. Our results suggest contralateral hippocampal microstructural abnormalities could be related to sustained seizures despite removing the pathologic hippocampus. Furthermore, utilizing this pre-surgical neuroimaging biomarker for TLE-HS network evaluation offers promise in assessing the risk of post-surgery seizure recurrence.

5.1.2 Functional connectivity of the temporal lobe and seizure outcome

In the *Study 2*, our focus shifted to the functional connectivity of the temporal lobe scheduled for resection and the rest of the brain. We explored how this connectivity might correlate with seizure outcomes. To achieve this, we employed the use of DC, a metric that captures both short- and long-distant connectivity. For each participant, we computed the DC value, which we then compared against the complete set of controls using the Crawford T-test, obtaining and individual T value for each patient. We then examined specifically the DC values of three temporal lobe regions (temporal pole, lateral temporal, and mesial temporal) (Figure 13).

Our comparison of patients to healthy participants indicated elevated wDC levels in the lateral temporal lobe, temporal pole, and mesial area for a majority of patients (Figure 14). Employing logistic regression, we showed that individual heightened DC in the temporal

pole and mesial area correlated with long-term seizure freedom post-surgery. The resulting model achieved a correct classification rate of 76.7% for patients, with greater accuracy observed in the SF group (Figure 15). A ROC curve analysis yielded an AUC value of 0.777 (Figure 16).

DC serves as a measure of intrinsic focal connectivity, highlighting regional hubs within the brain's functional network (Zuo et al., 2012). In epilepsy, patients often exhibit increased local connectivity in the epileptogenic area alongside decreased long-range connectivity (Englot et al., 2016). The measurement of local connectivity peaks has been linked to improved overall network topology following resection (Jackson et al., 2017). In the context of TLE, assessing connectivity patterns within the to-be-resected temporal lobe's subdivisions offers valuable insights into surgical prognosis. Specifically, elevated DC was associated with a favorable prognosis, suggesting strong local connectivity as a marker of region epileptogenicity and appropriateness for resection. TLE's diverse forms and outcomes accentuate the significance of evaluating individualized connectivity patterns, aiding in surgical decision-making. While MRI lesions guide surgical candidacy, additional modalities like PET contribute distinct prognosis-related patterns (M. L. Bell et al., 2009). Utilizing rs-fMRI can identify network anomalies, enhancing prognosis assessment before surgery.

5.1.3 Functional connectivity and cognitive outcome of surgery

Study 3 explored the connectivity along the longitudinal axis of both hippocampi: the one slated for surgery and its contralateral counterpart, dividing them in anterior and posterior regions. We examined their connectivity with three cognitive-related RSN – the DMN, DAN & EXE. We aimed to ascertain whether the connectivity between these networks and the anterior and posterior hippocampi correlated with reduced verbal learning, a common cognitive deficit among patients undergoing temporal lobe resections. We also

analyzed whether dividing the hippocampi in anterior and posterior divisions classified patients better than analyzing the connectivity of the hippocampi as a whole.

We employed MANOVA to compare the connectivity patterns of three groups: those with verbal learning decline after surgery, those with intact verbal learning after surgery, and controls. Specific connectivity differences were identified for the DMN and DAN with both the healthy and pathological hippocampus (Figure 21). Further analyses focused on anterior and posterior hippocampal connectivity with these networks. Substantial variations were observed, showing distinct patterns (Figure 22). Discriminant analysis aimed to differentiate groups using connectivity measures. For the whole hippocampus, the discriminant function distinguished controls from patients, but could not discriminate patients with verbal learning decline. However, using the in the anterior/posterior division of the hippocampus, the first function significantly differentiated learning-decline patients from other groups (Figure 23). The ROC curve was calculated to predict verbal learning decline based on significant connectivity variables. The results showed varying levels of AUC for different connectivity measures, with the anterior/posterior connectivity demonstrating the highest AUC (0.84), sensitivity (0.73), and specificity (0.95) (Figure 24).

As noted previously, cognitive functions arise from distributed brain networks. Resting state connectivity, particularly with the hippocampus and DMN, is crucial for cognition. Studies in TLE patients show altered network connectivity at rest and its associations with cognitive deficits after surgery, highlighting the importance and reproducibility of this approach (G. Doucet et al., 2013; G. E. Doucet et al., 2015; Mccormick et al., 2013). Our study found differences in anterior and posterior hippocampus connectivity with DMN and DAN, suggesting risk of cognitive decline after surgery varies based on network connectivity changes. TLE's heterogeneity and disrupted networks contribute to cognitive variations,

but analyzing hippocampus longitudinal axis connectivity with cognitive relevant networks might aid identifying surgery-related cognitive decline risk.

5.1.4 Functional connectivity and mood disorders after surgery

In *Study 4*, we assessed whether pre-surgical connectivity between the to-be resected hippocampus and the NAcc could enhance the risk of mood disturbances after surgery. Despite lacking prior psychiatric conditions, some patients experience mood decline post-surgery. Given that surgery disrupts crucial connections within the epilepsy and reward network, we investigated whether a pre-existing connectivity pattern might render patients vulnerable to post-surgical de novo depression.

To elucidate the functional NAcc, we used a functional MRI gambling paradigm, using a gain versus loss contrast (Camara, Rodriguez-Fornells, & Münte, 2009). In this contrast, both the control group and patients activated the reward network, including the NAcc on both resected and non-resected sides. Most patients (88.8%) displayed decreased NAcc-Hippocampus connectivity on the to-resected side when compared to controls. Patients with DnD and non-depressed nD patients both showed decreased connectivity compared to controls. The DnD group had significantly lower connectivity values than the nD group. An optimal cut-off point for post-surgical depression risk assessment was identified with an area under the curve of 0.87, sensitivity of 0.83, and specificity of 0.76 (Figure 27).

Having a shared underlying network of structures, the relationship between mood disorders and TLE is proposed as a bidirectional one, also highlighted by the high comorbidity between both diseases (B. Hermann et al., 2017; Vinti et al., 2021). In the case of TLE surgery, specifically in those patients with no prior psychiatric condition suggesting an intact reward network, the occurrence of depression raises the question of the impact of surgery in network connectivity. Our study investigated this connectivity, specifically between

NAcc and the to-be resected Hippocampus and found decreased connectivity as a potential risk factor for post-surgical depression.

However, only a subset of patients experienced depression, which could be tied to the preservation of other reward network structures and compensatory mechanisms on the contralateral side. This could explain the variability in outcomes. The etiology of depression is multifaceted, encompassing personal, social, and biological factors, and our study suggests a role for NAcc-Hippocampus connectivity. Our findings propose that NAcc-Hippocampus connectivity may contribute to triggering de novo depression after surgery, thus offering insights for pre-surgical counseling and tailored surgical approaches.

5.2 Integration of the results

The findings of our experiments underline the evolving perspective on TLE from a localized structural disorder to a complex neuronal network disease. These studies collectively illuminate the multifaceted landscape of TLE's pathogenesis, comorbidities, and response to treatment. By digging into the intricate web of biomarkers and connectivity patterns, these studies contribute significantly to our understanding of surgical prognosis and open avenues for more personalized therapeutic approaches.

All of the proposed biomarkers revolve around the interactions within the network of structures involved in TLE, as elucidated by Spencer (Spencer, 2002). For instance, assessing the extent of the seizure network, as evidenced by the microstructural architecture of the contralateral hippocampal subfields relative to the resection site and by assessing the connectivity of polar and mesial areas. Also, through obtaining the connectivity between hippocampus and key structures, as the RSN and the NAcc. These network interactions, measured by the proposed biomarkers, were susceptible to the influence of the surgical procedure, which in turn correlated with the different clinical outcomes of seizure freedom, verbal learning, and mood deterioration after surgery.

A fundamental line in this thesis is to consider the individual variability among patients with TLE. As outlined previously, averaging effects in a cohort of heterogeneous patients may overlook relevant, individual level effects (Lo et al., 2015). The heterogeneity observed in TLE arises from various factors, as discussed throughout this thesis.

Firstly, the use of multiple classification schemes in epilepsy and seizures has contributed to this heterogeneity. This is partly due to the evolving scientific understanding of epilepsy; it must be noted that till recently there was an ongoing debate as to whether epilepsy should be considered a disease or a disorder of the brain (Fisher et al., 2014). What is evident, however, is that there are numerous etiologies that can result in a similar disease

pattern, albeit with different underlying genetic and molecular epileptogenic mechanisms (Patterson et al., 2014; Pitkänen et al., 2014; Weber et al., 2014).

Secondly, the transition from individual neuronal behavior at the microscale to comprehending the dynamics of entire brain networks and the impact of epilepsy and surgery introduces a paramount level of complexity and individual variability. Studies examining the neurophysiological behavior of neuronal populations at the microscale have revealed intricate interactions involving both synchronization and desynchronization among neuronal clusters, which may vary in spatial proximity and may affect normal, non-pathologic neuronal tissue (Feldt Muldoon et al., 2013b; Jiruska et al., 2013; Szabo et al., 2015). Consequently, this network behavior at the microscale traduces unique patterns in seizure signatures when studied at larger neuronal populations, such as those typically available in clinical settings (Bartolomei et al., 2017; Chauvel et al., 2014).

Lastly, at the broader scale, when investigating larger or whole-brain connectivity with techniques such as rs-fMRI, one can observe a distinct reorganization in neuronal connectivity and function at the individual, single subject level (Bernhardt et al., 2019; Bonilha, Jensen, et al., 2015). Besides, temporal lobe surgery plays a significant role in the treatment of these patients. Rather an aggressive treatment, it can be curative in well-tailored patients. The network biomarkers analyzed serve as a bridge between these two ends, connecting individual network complexity to a more radical treatment approach that can be tailored effectively to the needs of the individual patients.

Our results integrate in this frame; being at the end of the scale of complexity, provide a window in the individual differentiation of pathologic epileptic networks, emerging from a common substrate of neural dysfunction due to the chronic epileptogenic process. Particularly in the case of *Studies* 1 & 2, were a connection between microstructural architecture

and network connectivity of individuals with TLE could be established with their response to surgery in terms of seizure outcome.

The hippocampus, a structure commonly affected in TLE, serve as a neuronal hub (Strange et al., 2014), and its interactions are the key for understanding its role in cognitive and emotional functions (Bullmore et al., 2009, 2012; A. R. McIntosh, 2004). Remarkably, a degree of network readaptation can be observed in epileptic patients, which may contribute to sustaining their functioning, as for instance the function of the contralateral hemisphere (Bettus et al., 2009; Larivière et al., 2020). However, this readaptation can be quite fragile (Van Diessen et al., 2014), and external factors such as surgery can easily disrupt it, resulting in a loss of function (G. E. Doucet et al., 2015; Rodriguez-Cruces et al., 2022). In *Study* 3 we discovered a specific connectivity pattern in patients with increased anterior hippocampal connectivity but decreased posterior hippocampal connectivity to the RSN. These patients were found to be at a higher risk of experiencing a decline in verbal learning compared to other patients. This finding aligns with previous research, which suggests that increased activation of posterior hippocampal regions during encoding tasks can protect against verbal impairment after surgery (Bonelli et al., 2010). Conversely, in *Study 4*, patients who experienced mood decline already exhibited decreased hippocampal-NAcc connectivity. This raises the question of whether a higher level of hippocampal adequacy is a factor in this case, being the disconnection of this relation what could trigger the advent of mood decline.

Finally, there is accumulating evidence of the continuum beneath structural alterations and functional disruptions seen in TLE (Larivière et al., 2020). Recent evidence have introduced the notion that diminished microarchitectural distinctions within the temporal lobe and other paralimbic regions compared to the rest of the cortex offer a tangible foundation

for the functional network reorganization and consequent cognitive impairments characteristic of TLE (Royer et al., 2023). Our findings in microstructural alteration of the hippocampus and also its connectivity represent this continuum of pathologic findings.

Collectively, these experiments remark the potential benefits of adopting a network-oriented approach when counseling patients undergoing epilepsy surgery. Moreover, insights gleaned from non-invasive functional and diffusion MRI techniques illuminate a host of pertinent markers, from microstructural abnormalities to network reorganization, that contribute to our understanding of these seizure, cognitive and mood outcomes of surgery.

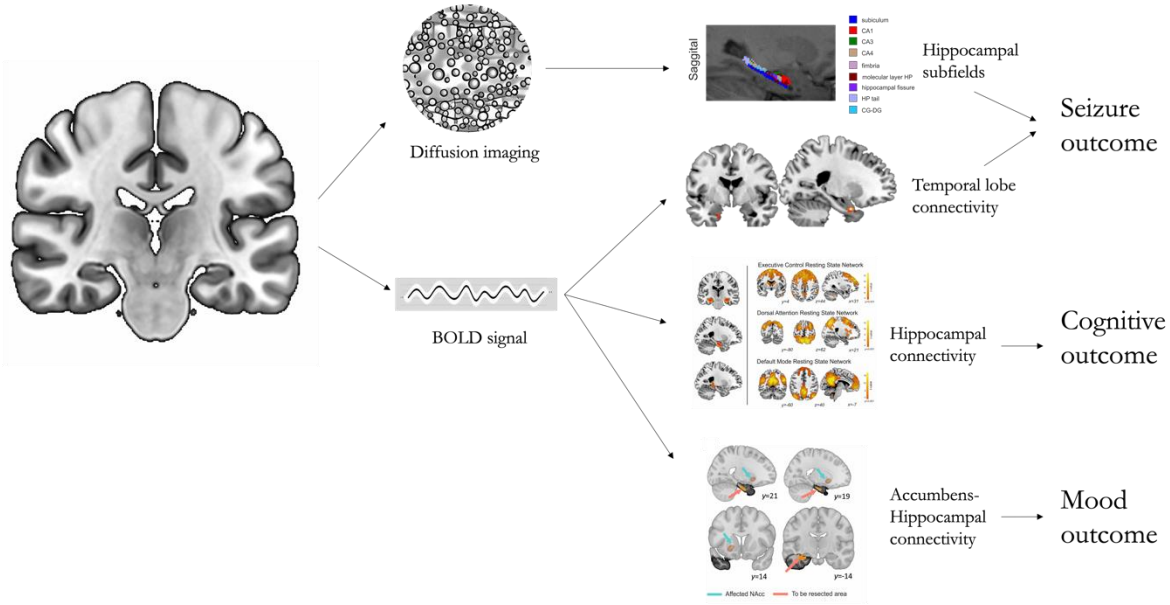


Figure 28. Schematic view of the 4 biomarkers studied in this thesis and its applications.

5.3 Clinical applications

Given the potential heterogeneity encompassed within TLE, as indicated by previous research (Bonilha, Martz, et al., 2012; Malmgren et al., 2012), the identification of markers indicative of a favorable surgical response is of paramount importance for clinicians. In light of this, we established different relationships between several network-related parameters and surgical outcome.

A pivotal aspect in the management of TLE involves an accurate assessment of the extent of the epileptogenic zone. This determination play a crucial role regarding the candidacy for temporal lobe resection, the necessity of intracranial evaluation, and the establishment of a prognosis. While the presence of a detectable lesion on MRI is a key factor guiding surgical interventions in TLE, it is crucial to recognize that a subset of patients with confirmed lesions may still experience relapses. Conversely, some patients with unremarkable MRI results may benefit significantly from surgical intervention (Ryvlin et al., 2016).

Beyond the binary consideration of MRI-based lesion presence, insights from studies exploring network extension have significantly contributed to individual prognosis. For instance, the occurrence of frequent secondary generalized seizures is a biomarker indicating a worse prognosis for epilepsy surgery (A. M. McIntosh et al., 2004). It is well established that secondary generalized seizure engage larger networks compare to cases with absence of generalization (H. Blumenfeld et al., 2009), and stronger interhemispheric connectivity, related to seizure propagation patterns, is related to larger degradation of microstructural integrity (Miró et al., 2015).

Besides, the integration of nuclear medicine imaging has unveiled distinct prognostic outcomes. These insights provide valuable guidance for clinical decision-making, and are nowadays in widespread use in epilepsy units around the world (M. L. Bell et al., 2009; Carne et al., 2004).

Importantly, our study contributes to the potential of DTI and rs-fMRI to identify critical network alterations that could significantly enhance the accuracy of prognostic assessment preoperatively (Bonilha, Jensen, et al., 2015; Lee et al., 2022; Q. Li et al., 2021). This innovative approach in neuroimaging has the capacity to improve the evaluation of prognosis prior to surgical intervention, improving our understanding of TLE and its intricate neurophysiological underpinnings.

Another crucial aspect in determining surgical candidacy is the risk of undesirable sequelae (Doherty et al., 2021; C. Helmstaedter et al., 1996). Traditionally, cognitive deficits have been recognized as significant "side effects" of surgery (B. Bell et al., 2013), and now, increasingly, mood disorders are gaining prominence as well (Wrench et al., 2011b). These post-surgical effects can substantially diminish the quality of life after the procedure, potentially overshadowing a positive seizure outcome. As highlighted throughout the thesis, various approaches over the years have been employed to quantify cognitive functioning and assess the involvement of structures in TLE, especially those undergoing resection during surgery (B. Bell et al., 2013; B. Hermann et al., 2017).

The biomarkers proposed in this thesis, derived from non-invasive rs-fMRI, have the potential to enhance pre-surgical decision-making. By attempting to evaluate the risk of cognitive decline and mood disorders, these biomarkers can contribute to improved counseling for individual patients regarding the potential unwanted effects of surgery.

Moreover, both DTI and rs-MRI techniques are currently widely available, harmless, and non-invasive for patients. These methods can be integrated with standard MRI sequences already currently performed in the presurgical evaluation of patients with TLE. These characteristics add significant value in terms of clinical application, offering a safe and accessible means to enhance the assessment of TLE surgical prognosis.

5.3.1 Individual level analysis

One of the foremost considerations when evaluating a biomarker for predicting the prognosis of epilepsy surgery is its validity at the individual level. In alignment with the concept of disease heterogeneity, we focused the clinical significance of our biomarkers by assessing its relevance in the context of individual subjects.

In studies 2 and 4, we used a Crawford-T test to obtain the value at individual level by comparing each patient against the control group. The Crawford t-test, also known as the Crawford's modified t-test, is a statistical method used to compare the performance of a single participant or case to a normative group or control group (J. Crawford et al., 1998). It is commonly employed in neuropsychological and clinical research to assess whether an individual's test scores significantly differ from those of a reference group. This method provides a robust approach for making statistically valid inferences about individual cases, even in situations where the data might not conform to normal distribution assumptions (J. Crawford et al., 1998). This approach aimed to derive individual connectivity values that reflect how distinct a single patient is from our normative group. Once these individual T-values were obtained, we subjected them to the desired effect testing through ROC analysis.

Across all conducted experiments, the ROC analysis was employed as a pivotal methodology. The primary objective of employing ROC analysis was to discern optimal cut-off points, facilitating the evaluation of the specificity and sensitivity characteristics inherent to the identified markers (Obuchowski et al., 2018).

The ROC analysis is a widely recognized statistical technique used to evaluate the performance of a model in distinguishing two classes, typically the true positive rate against the false positive rate across various threshold values. This graphical representation provides a comprehensive depiction of the discriminatory capacity of the markers. By systematically

altering the threshold, the ROC analysis evaluates how the sensitivity (true positive rate) and specificity (true negative rate) trade off against each other.

Through the ROC analysis, the establishment of an optimal cut-off point is guided by the aim of attaining the highest achievable sensitivity and specificity concurrently. The point on the ROC curve nearest to the upper left corner signifies the threshold that maximizes the diagnostic accuracy of the marker. This point effectively represents the ideal equilibrium between correctly identifying positive cases and accurately excluding negative cases.

Furthermore, the AUC serves as a quantification of the overall discriminatory power of the marker, with an AUC value of 1 indicating perfect discrimination and a value of 0.5 denoting a performance equivalent to random chance. Consequently, the utilization of ROC analysis within these experiments ensured a rigorous assessment of the markers' discriminatory potential, thereby enhancing the precision and reliability of our findings.

5.4 Limitations and future directions

5.4.1 Global limitations

The presented experiments are marked by two central limitations that impact the broader generalizability and practical applicability of our findings. These limitations warrant careful consideration and a nuanced understanding of the potential boundaries within which our results can confidently be extrapolated and applied.

Firstly, the size of our patient cohort across the four experiments is modest. Despite yielding statistically significant findings, the modest number of participants and the uneven distribution of effects being studied – namely, seizure freedom, cognitive outcomes, and mood disorders – underscore the need for caution when attempting to generalize the biomarkers revealed in these experiments. It's important to note that the statistical thresholds employed in fMRI research have progressively become more conservative to minimize Type I errors (false positives). However, this unwavering emphasis on minimizing Type I errors inadvertently leads to heightened Type II errors (missing genuine effects), a bias towards investigating large rather than small effects (Lieberman et al., 2009).

Power analyses suggest that the ongoing reduction in acceptable P-values is resulting in substantial increases in the Type II error rate. Moreover, the push for a map-wide FDR of 0.05 is based on the assumption that this mirrors the FDR in most behavioral research. However, this perspective misrepresents the prevailing conventions in actual behavioral research. Simulations have demonstrated that a combined threshold involving both intensity ($p< 0.005$) and cluster size (with a 10 voxel extent) strikes a desirable equilibrium between Type I and Type II error rates. This combined thresholding method yields elevated yet acceptable Type II error rates and achieves an FDR comparable to the effective FDR observed in typical behavioral science articles. (Lieberman et al., 2009)

Considering that our intention in these experiments is to identify novel biomarkers, our chosen approach is justifiable, but its validity demands replication. Type I errors are self-correcting in nature since they fail to replicate, thus permitting a more lenient thresholding strategy to avert Type II errors.

Secondly, it is crucial to address the approach employed for the analysis and extraction of fMRI and DTI biomarkers. This process involved the utilization of various software packages, each providing distinct functionalities for data processing. While many of these software packages are readily accessible, it's imperative to acknowledge that their effective utilization demands a nuanced understanding of their functionalities, often necessitating the creation of customized scripts or programming scripts. Even though these scrips can be shared, the intricacy of the analysis pipelines can pose a potential barrier to seamless replication of the results and application in a wide clinical setting. In any case, we specified the methods used and tried to ensure the reliability and reproducibility of our results.

Moreover, it is important to underscore that the suitability of these analysis software packages for clinical employment has not been definitively established. This notable limitation accentuates the complexity of replicating and subsequently validating the biomarkers under investigation. The absence of formal validation for clinical utility introduces an additional layer of intricacy in the replication process, further challenging the comprehensive validation of the studied biomarkers. This consideration highlights the importance of adopting a cautious approach when interpreting the findings and emphasizes the need for robust validation strategies in subsequent research endeavors.

Lastly, across three experiments, we lacked post-surgical imaging to monitor the extent of resected tissue and investigate alterations in network parameters following the surgery. This aspect holds importance when assessing seizure outcomes, as the removal of specific

tissue and the extension of the lesioned tissue play a pivotal role in prognosis (Hennessy et al., 2000; Simon S. Keller et al., 2017). Moreover, analyzing post-surgical resting state and DTI data holds significant value in examining the post-surgical readaptation of network parameters, shedding light on network plasticity and aiding our comprehension of the surgical impact on our biomarkers (Bonelli et al., 2013). This limitation was addressed in study 4, where we used post-surgical resection masks to study the pre-operative connectivity of the to-be resected tissue. Finally, we did not take into account the hemispheric dominance of each subject, as this fact has implications in connectivity and cognition (B P Hermann et al., 1997; Miró et al., 2014). As a counterpart, even though we considered as pathological/non-pathological groups, we analyzed in each experiment whether lateralization of surgery had an impact on the effect, without significant differences (Figures 8, 12, 18 & 26).

5.4.2 Limitations by experiment

Each experiment also has specific limitations. In *Study* 1, the focus was on patients with TLE-HS. Although the findings established a link between hippocampal microstructure contralateral to resection and long-term seizure freedom, we did not have a pathology analysis of the specimen to contrast our findings. Moreover, the HS subtype was not delineated in the resected tissue or on the MRI scans, the latter due to methodological constraints that restrict the application of segmentation software in severely damaged hippocampi.

Study 2 delved into TLE surgical patients, but most of the sample had MRI with lesional HS. The study's reliance on its own patient cohort, skewed towards this specific condition, highlights the need for broader inclusion to enhance the generalizability of results for the whole TLE spectrum. The presence of patients with normal MRI and normal pathology increases the potential clinical significance of the findings but still further validation is required. Also, the biomarker studied, DC, did not differentiated between specifically long-range and short-range connectivity, which apparently is a signature of the epileptogenic

network (Jackson et al., 2017). Finally, a lack of control for resected tissue of the temporal lobe diffuses the validity of the findings.

Study 3 explored memory impairment post-surgery. Again, no correction for tissue resected was applied, particularly the extent of resection of mesial and lateral temporal lobes which influences cognitive outcome (B. Hermann et al., 2017), indicating the necessity of accounting for these factors in further studies. Moreover, again the high numbers of patients with TLE and HS might limit the findings' applicability to this subgroup. Ultimately, we did not distinguish patients based on their dominant or non-dominant hemispheres; instead, we controlled for lateralization in our analysis even though it can be relevant for verbal functioning and may imply a network reorganization (Miró et al., 2014).

In *Study* 4, the focus shifted to mood disorders, specifically investigating the potential of Nacc-Hippocampus connectivity as a biomarker of mood decay after surgery. Despite significant findings, the omission of considering other social factors impacting mood disorders, such as marital status or employment, highlights a potential avenue for future research. Further, evaluating hippocampal function with a more specific task, such as a task evaluating reward mediated learning (Vilà-Balló et al., 2017) could generate further insight in the pathology of mood disorders in TLE. Finally, we did not evaluate the amygdala complex connectivity, even though it is a relevant structure in mood disorders and is affected in TLE (Kemmotsu et al., 2014).

5.4.3 Future directions

Current research in temporal lobe epilepsy underscores the significance of delving into inter-patient heterogeneity, acknowledging it as a pivotal stride towards adopting a person-centered approach to care. (Lee et al., 2022). Besides, the acknowledgment of epilepsy as a

network disease and the utilization of this framework have been widely embraced to enhance our comprehension of the condition (Bernhardt et al., 2019; Larivière et al., 2020; Q. Li et al., 2021; Rodríguez-Cruces et al., 2020; Royer et al., 2023).

The practical application of our findings is anticipated, as previously mentioned, due to the necessity for a more substantial cohort of patients to be encompassed. Consequently, one of the forthcoming phases involves subjecting these identified biomarkers to testing within more large sample groups, adopting a prospective methodology. Additionally, it is imperative to consider the evaluation of resected tissue: both pre-surgery, with the intention of establishing a predictive model, and post-surgery, to validate alterations in network markers subsequent to the surgical intervention, with a particular emphasis on longitudinal investigations for dynamic changes of whole-brain network topology (Jackson et al., 2017).

Furthermore, a multifaceted approach that incorporates all network-related biomarkers into a multidomain predictive model holds intrinsic appeal, given the discernible degree of intercorrelation among the investigated biomarkers. Incorporating seizure, cognitive, and emotional outcomes into a multivariate, data-driven analysis could provide the opportunity to discern various clinical profiles and improve personalized counseling strategies. This strategy is particularly pertinent as all the biomarkers are inherently linked to MRI data and are intrinsically associated with connectivity, whether functional or structural.

Finally, a more comprehensive work-out of seizure outcome, and cognitive and psychiatric profiles is needed in order to distinguish further patterns in network reorganization. Rather than employing a discrete, categorical division with a simple yes or no among patients, enhancing the classification of outcomes would significantly improve prognostic assessments. In the context of seizure outcomes, numerous classifications aim to encompass the complexity, considering that seizures may reappear after surgery at varying intensities

(De Tisi et al., 2011; J. J. Engel et al., 1993; H. G. Wieser et al., 2001). For cognitive outcomes, as mentioned earlier, can be assessed through a diverse range of tests. However, considerable efforts have been dedicated to refining classifications that capture the intricate nuances of evaluated cognitive functions, striving for increased replicability (Reyes et al., 2019; Vogt et al., 2017). Regarding to mood disorders, few studies used standardized tools to assess mood and anxiety (Doherty et al., 2021; Ramos-Perdigués et al., 2018). Besides, investigating the role of the hippocampus during a reward task could enhance our knowledge of its role within the network, and thus increase the understanding of the relevance or not of its resection (Vilà-Balló et al., 2022). Finally, evaluating quality of life parameters through standardized scales would also be relevant for a comprehensive assessment of post-surgical outcomes on a global scale (Dupont et al., 2006; Jerome Engel, 2012; Vakharia et al., 2018).

Certain facets of our research have already been set in motion; comprehensive analysis of structural connectivity, quantified through DTI has already demonstrated its significance in prognosticating outcomes following temporal lobe surgery (Bonilha, Jensen, et al., 2015). Additionally, more recent endeavors focusing on functional connectivity using rs-fMRI have also exhibited the capacity to prognosticate surgical outcomes (Larivière et al., 2020). Building on the insights from prior studies, the underlying foundation of these network alterations has been proposed to stem from the microstructural modifications in the superficial white matter of limbic regions, which have been related to consequential large-scale network changes (Bernhardt et al., 2019; Liu et al., 2016). This association between limbic microstructure and extensive functional network modifications has, in turn, been linked to cognitive implications (Royer et al., 2023).

Finally, to enhance the reliability and validity of the investigations, collaborative efforts have been fostered on an international scale in order to obtain large datasets. These initiatives are geared toward augmenting the availability of DTI and rs-fMRI data in the epilepsy domain. Notable endeavors include the ENIGMA cohort, which is actively assembling an extensive database of epilepsy patients to enhance the reproducibility of studies (Sisodiya et al., 2022), and the Human Connectome Project, an endeavor aimed at comprehensive mapping of human brain connectivity (Glasser et al., 2016). These collaborative ventures are pivotal in advancing our understanding of epilepsy and its neural underpinnings.

5.4.4 Final remarks

This thesis represents a distinctive approach to comprehending epilepsy surgery for TLE, viewing it through the lens of a network-based disorder. Within this framework, by examining various biomarkers and connectivity patterns, the studies offer insights into surgical prognosis and advocate for a more personalized therapeutic approach. The findings emphasize the significance of individual variability in TLE and the potential benefits of adopting a network-oriented approach in counseling patients undergoing epilepsy surgery.

Remarkably, the biomarkers were derived from existing MRI sequences that are readily implemented, albeit currently underutilized in clinical settings. These non-invasive sequences can be safely administered to patients in outpatient settings and seamlessly integrated into other MRI protocols. Finally, our patient cohort had a comprehensive, long-term follow-up, lending heightened validity to the outcomes assessed, particularly in the context of seizure outcomes.

In Study 1, we explored the microstructure of the contralateral hippocampus to the resected side and its association with post-surgical seizure freedom. This investigation allowed us to establish a connection between functional connectivity within the TLE network

and the surgical outcome. In Study 2, we introduced a novel functional connectivity biomarker known as DC, which exhibited a significant correlation with post-surgical seizure freedom. DC serves as a comprehensive measure of both short and long-distance connectivity, and we assessed it at the voxel level for each individual, revealing its relevance to surgical outcomes. In Study 3, we observed variations in connectivity between the hippocampi and resting-state networks associated with cognition. These differences were evident before surgery among patients who experienced a decline in verbal learning following resection, as opposed to those who did not encounter such declines. In Study 4, we identified a notable reduction in connectivity between the Nucleus Accumbens and the hippocampus slated for resection. This decrease was found to be statistically significant in patients who later developed post-surgical de novo depression.

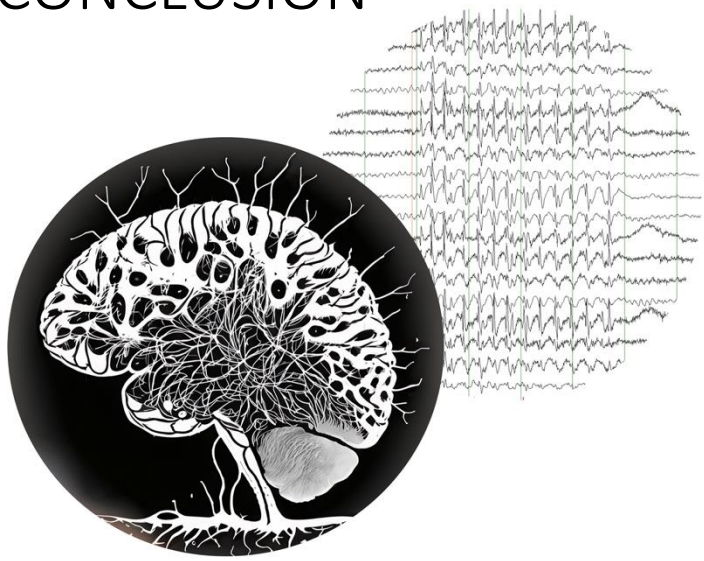
These experiments are contextualized within the framework of the network-based hypothesis of brain functioning. Emphasizing the interactions among neuronal populations, they underscore the pivotal role these interactions play in the emergence of normal and pathological brain function. As uncovered, substantial network alterations, whether they are large in scale or involve network nodes, play a crucial role in determining the prognosis of surgery for TLE in terms of seizure freedom, verbal learning, and mood decay. The biomarkers proposed in the studies shed light on how epilepsy and surgical interventions can disrupt networks, potentially leading to the emergence of various symptoms.

In our comprehensive research endeavor, we have meticulously evaluated each of the suggested biomarkers at the level of individual patients. This meticulous approach was essential to confront and manage the inherent variability and diversity seen in temporal epilepsy cases, especially when compared to the relatively uniform resection procedure. Our objective has been to move beyond the one-size-fits-all paradigm and to foster a more per-

sonalized and patient-centric approach. By doing so, we aim to empower healthcare practitioners with the tools and insights necessary to make informed decisions that are tailored to each patient's unique needs and circumstances.

This personalized approach acknowledges that temporal epilepsy is a complex and multifaceted condition, and its surgical management should reflect the individual characteristics and nuances of each patient's condition. By incorporating these biomarkers into our decision-making process, we aspire to optimize surgical treatment outcomes and enhance the overall quality of care for individuals living with temporal epilepsy.

CONCLUSION



6 CONCLUSIONS

1.- In this thesis, we identified four biomarkers derived from a network-based approach to TLE, utilizing DTI and rs-fMRI.

2.- These biomarkers yielded valuable insights into surgical prognosis, particularly regarding seizure control, cognitive function, and mood outcomes in patients with TLE.

3.- Our studies are framed within the network-based hypothesis of brain functioning, highlighting the role of network alterations in surgical outcomes.

4.- Our findings emphasize the potential for a patient-centric approach in managing temporal epilepsy, optimizing treatment outcomes and enhancing overall care quality.

5.- Biomarkers are derived from widely available MRI sequences so they can be easily integrated into clinical settings, and they are non-invasive and harmless.

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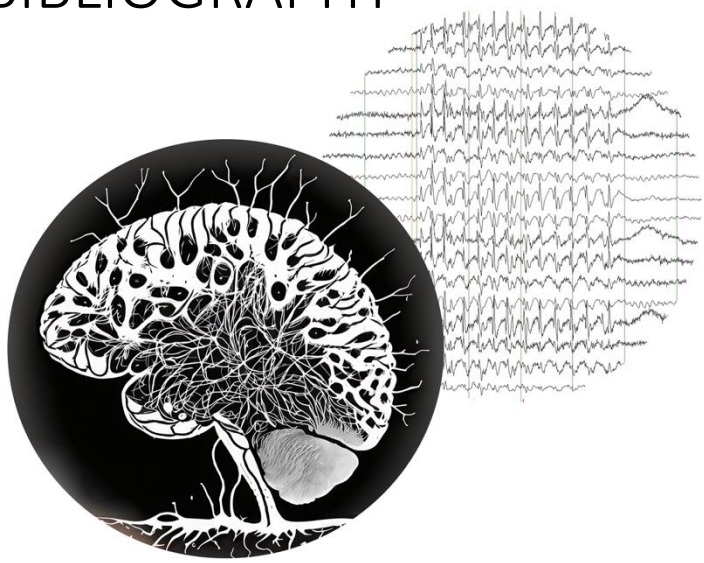

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