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Structural and functional MRI alterations in patients with Temporal Lobe Epilepsy eligible for surgery: a study on the contribution of non-invasive neuroimaging techniques to surgical planning.

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ABBREVIATIONS

ACC: anterior cingulate cortex

AEDs: anti epileptic drugs

ASL: arterial spin labeling

DMN: default mode network

DNET: dysembryoplastic neuroepithelial tumor

DTI: diffusion tensor imaging

EEG: electroencephalography

FC: functional connectivity

FCD: focal cortical dysplasia

fMRI: functional magnetic resonance imaging

FS: febrile seizures

HS: hippocampal sclerosis

IED/IEDs: interictal epileptiform discharge/s

MCD: malformations of cortical development

MRI: magnetic resonance imaging

PET: positron emission tomography

PLNTY: polymorphous low-grade neuro-epithelial tumor of the young

PMG: polymicrogyria

PNH: periventricular nodular heterotopia

PPC: posterior cingulate cortex

TLE: temporal lobe epilepsy

CHAPTER 1: BACKGROUND

Temporal lobe epilepsy (TLE), the most common type of focal epilepsy, is a syndrome characterized by seizures arising from the cerebral structures within the temporal lobes. Its natural history is represented by frequent seizures with impairment of consciousness, often resistant to antiepileptic treatment, associated to reduction in quality of life and increment in morbidity and mortality (Sperling et al., 1996).

The surgical treatment in these patients can interrupt seizures, leading to clinical, psychological and social improvement (Engel et al, 1999; Wiebe et al., 2001). Antero-medial temporal lobectomy is the main type of resective surgery for TLE associated to hippocampal sclerosis (HS), although over the years different surgical techniques have been used in these patients (from selective amygdalo-hippocampectomy to less invasive techniques like MRI-guided thermo-ablation of the hippocampus) (Asadi-Pooya et al., 2017). Since the first record of temporal lobectomies performed by Penfield and Flanigin in 1950, several studies have reported series of surgical treatments of intractable TLE achieving variable outcomes (40-85%) in terms of seizure freedom (Sperling et al., 1996; Wiebe et al., 2001, Engel et al., 2012).

One of the most important steps in the history of TLE surgery was the identification of clear epileptogenic lesions localized in the temporal lobes on brain MRI, such as hippocampal sclerosis. In particular a subtype of TLE with febrile convulsions in early childhood, HS on MRI, and favourable post-surgical outcome, has been identified as a surgically remediable syndrome (Berkovic et al., 1991).

A recent histopathological study (Blümcke, et al., 2017) on more than 9500 resected brain specimens obtained by epilepsy surgery for drug-resistant seizures in 36 European Centres over 25 years, demonstrated that hippocampal sclerosis was the most common aetiology found in focal epilepsy in general, and accounted for the 54.4% of the

specimens obtained by the temporal lobes. In 1.5% of cases a second diagnosis was associated to HS. Tumours and malformations of cortical development (MCD) represented the second and third most common causes of focal epilepsy.

In a minority of patients with typical electro-clinical TLE no clear structural lesion can nevertheless be observed on MRI. Often these patients need a more extensive pre-surgical workup that include intracerebral EEG recording to define the epileptogenic zone. Different studies have shown that surgical outcome in these cases is less favourable (Berkovic et al., 1995; Berg et al., 1998).

Since the beginning of years 2000 non-invasive neuroimaging procedures (functional MRI, co-registration of EEG with fMRI, cerebral 18-FDG PET, High-Density EEG, Magnetoencephalografy, etc) have been being more and more used as part of the pre-surgical diagnostic process, with variable effects on the final outcome.

New insights into the complexity of neuroanatomical and neurofunctional aspects of the temporal lobes, and their connectivity in the whole brain, have been reached through these techniques and may represent a guide for surgery.

CHAPTER 2: Design of the study

An observational prospective multi-centric clinical and neuroimaging study was conducted in adolescent and adult patients with drug-resistant TLE eligible for surgery, in a 3-years period (2016 – 2019).

The Italian network project was titled "Magnetic Resonance Imaging in drug-refractory temporal lobe epilepsy: standardization of advance structural and functional protocols at 3T, to identify hippocampal and extrahippocampal abnormalities."

The network project's rational derives from the "Italian League Against Epilepsy Neuroimaging Commission" with the purpose to define a standardized structural and functional 3T MRI protocol in patients with temporal lobe epilepsy.

Four 3rd level Epilepsy Centres participated ("C. Mondino" National Neurological Institute in Pavia; Neurological Institute "Carlo Besta" in Milano; Epilepsy Centre at Neuroscience Department, University of Modena and Reggio Emilia; "Claudio Munari" Epilepsy Surgery Centre, Niguarda Hospital, Milano).

Three working-packages (WP) were identified:

- WP1: comparison between 3T structural MRI and a) 1,5T structural MRI b) 3T microstructural data (Diffusion Tensor Imaging or DTI) c) Arterial Spin Labeling (ASL) perfusion data

-WP2: comparison between 3T structural MRI and a) histopathology results from epilepsy surgery specimens b) ex-vivo 7T MRI data.

-WP3: comparison between 3T structural MRI and functional MRI data (EEG-fMRI co-registration).

AIMS OF THE STUDY

1) To identify hippocampal and extrahippocampal abnormalities in the temporal lobe through a standardized structural and functional 3T MRI protocol.

For patients undergoing epilepsy surgery validation of the protocol was obtained comparing radiological data with histopathological findings (gold standard), with 7T MRI data of surgical specimens (WP2), and with postsurgical outcome on seizure freedom.

2) To verify the diagnostic power of advance functional MRI techniques such as DTI and ASL (WP1) and simultaneous EEG-fMRI co-registration (WP3).

Microstructural and functional abnormal areas were compared to macrostructural abnormalities on 3T MRI, gold standard histopathological data, and post-surgical outcome.

3) In the group of patients studied previously with 1,5T structural MRI, neuroimaging data were compared with high field 3T MRI to verify diagnostic power of 3T MRI (WP1).

METHODOLOGICAL ASPECTS

Patients older than 12 years of age with electro-clinical diagnosis of drug-resistant temporal lobe epilepsy were consecutively enrolled at the 4 Italian Epilepsy Centres from December 2016 to December 2019.

WP1 was set in Pavia; WP2 was set in Milano, and WP3 in Modena.

For Pavia and Modena enrolment of 10 patients/year was expected.

For Milano (“Carlo Besta” Neurological Institute and “Claudio Munari” Epilepsy Surgery Centre at Niguarda Hospital) enrolment of respectively 20 and 40 patients per year was expected.

The total number of enrolled patients expected for the multicentric network project was 250.

All patients performed an extensive pre-surgical evaluation inclusive of a share standardized structural and functional 3T MRI protocol.

Moreover, a share database with historical, clinical, neurophysiological and radiological data was created.

The present thesis is focused on the electro-clinical data obtained by the shared database, on the results of standardized structural MRI protocol and on the analysis of functional data derived from simultaneous EEG and fMRI co-registration (WP3).

CHAPTER 3: Electro-clinical findings in TLE

Two hundred and fifty-five patients (132 females and 123 males) with drug-resistant TLE eligible for surgery agreed and signed the informed consent and were therefore enrolled in the study.

A hundred patients were enrolled in Milano-Niguarda Hospital, 63 patients in Milano-Besta Institute, 49 patients in Modena and 43 patients in Pavia.

METHODS

Information from the share database was analysed.

Items from history included hand dominance, family history for epilepsy, neurological insults in the past, febrile convulsions in early childhood, neurological examination, age at epilepsy onset, duration of epilepsy, seizure frequency, type of seizures, presence of status epilepticus or falls, seizure clusters, influence of circadian rhythm, psychiatric comorbidities and drug therapy in the past and present.

Data obtained from video-EEG telemetry were also collected, focusing on ictal semiology and type and localization of interictal and ictal EEG abnormalities.

RESULTS

Clinical history

The main data on epilepsy history are summarized in Table 1.

The majority of patients (82%) were right-handed (n=210 patients). Family history for epileptic seizures was reported by the 17% of patients (n=44), while history of febrile seizures (FS) in early childhood was present in the 16% of the patients (n=42). Out of

these patients, 26 had simple FS, 15 had complex FS, and one patient reported to have both simple and complex FS.

In 96% of patients (n=246) the neurological examination showed no alterations.

Personal antecedents were present in the 18% of patients (n=47), and were represented by threatened miscarriage (n=1), birth dystocia (n=5), perinatal distress (n=15), CNS infections (n=13), head injury (n=10) and previous brain surgery (n=3).

Psychiatric history was reported by 18% of patients (n=46): anxiety (n=24) and depression (n=21) were the most frequent disorders reported.

All patients at enrollment were on medical treatment with antiepileptic drugs (AEDs): 30% (n=76) of patients were taking only one AED, 40% (n=104) were taking a bi-therapy, and 30% (n=75) were taking more than 2 AEDs. In reviewing the pharmacological history it appeared that only 18% of patients were taking the same therapy since the epilepsy diagnosis. In the remaining patients, one AED was already withdrawn (23%), while in the majority of patients (58%) more than 1 AED was already withdrawn due to ineffectiveness.

Table 1: Clinical and demographical data in 255 TLE patients.

	Mean	**Range**	**Median**
Age at study enrollment	36.6 years	14-66 years	37 years
Age at epilepsy onset	19.6 years	1-61 years	17 years
Duration of epilepsy	17.3 years	1-43 years	16 years
Seizure frequency	7.9 sz/m	0-30 sz/m	3 sz/m
	N pt	**%**	
Seizures during wakefulness	215	84	
Seizure-free periods*	73	29	
Cluster seizures	95	37	
Focal aware seizures	123	48	
Focal impaired awareness seizures	225	88	
Focal to generalized seizures	124	48	
Status Epilepticus	1	0.4	
Falls during seizures	57	22	

Sz/m= number of seizures per month. N pt= number of patients *: Period longer then 1 year without seizure recurrence.

Video-EEG monitoring

EEG was performed in 97% of the patients. Interictal epileptiform discharges (IEDs) were represented by paroxysmal events such as spikes, spike-and-waves, fast activities (present in the 86% of patients), and slow abnormalities (theta or theta-delta slowing), recorded in the 88% of patients. In 73% of patients IEDs were unilateral. In the remaining cases, 10% were represented by synchronous IEDs spreading contralaterally, and 17% were asynchronous IEDs.

The most frequent ictal onset on EEG was the occurrence of rhythmic theta/delta activity on temporal derivations, recorded in 29% of patients. In 22% of patients seizure onset was associated to focal fast activity, and in 16% to desynchronization of EEG.

Spike bursts, reduction in slowing, or not clearly visible onset were the remaining types of seizure onset.

Auras were present in 77% of patients: the most frequent was epigastric aura (32%), followed by psychic (27%) and dysautonomic (7%). Frequent ictal semiology signs were represented by impairment of awareness (85%), oral automatisms (47%), and limb dystonia (26%).

DISCUSSION

In the present observational multi-centric Italian study, electro-clinical findings from 255 consecutive patients with TLE eligible for surgery were investigated; a "state of the art" of this condition nowadays is reviewed. Our data confirm that TLE is a focal epilepsy syndrome of the adult age, with a mean seizure onset age of 19.6 years and a long disease history (mean: 17.3 years). These results are similar to previous series on drug-refractory TLE patients in the United States (Sperling, et al., 1996; Wiebe, et al., 2001) and in Italy (Tassi et al., 2009), dating back to 20 or 10 years ago, showing that patients with TLE still receive surgery after many years of seizures recurrence, despite the effort in promoting early surgical intervention. A reason might be that patients experiencing temporal lobe seizures refer late to epilepsy clinics, due to the typical seizure semiology, which can be underestimated by the patients themselves, or confounded with psychosomatic disturbs. Moreover, programs of epilepsy surgery are not available everywhere in the Country, therefore 3rd level Epilepsy Centres share long waiting list before surgical intervention.

The finding of drug-resistant seizures, with a recurrence on a weekly base (mean seizures/months: 7.9; median seizures/month: 3), is also in line with previously reported

series (Tassi et al., 2009, Engel et al., 2012). According to the new ILAE Classification of Seizures (Fisher, et al., 2017) focal awareness impaired seizures were the most frequent type of seizure, observed in up to 88% of the patients; focal aware and focal to generalized seizures were present in almost half of the cases, while status epilepticus was extremely rare, in fact only a patient reported this condition in his history.

History of febrile seizures in early childhood was present in the 16% of the patients; this data is lower than previously described series in which 30-35% of patients reported FS in medical history (Tassi et al., 2009; Asadi-Pooya et al., 2016). A possible explanation of this reduction might be related to a wider awareness of this condition, and a better way of treating it compared to previous years. In particular fast treatment of febrile convulsions can lead to prolonged seizures prevention (Dubè et al., 2007). Febrile seizures and later development of hippocampal sclerosis, is a well-known association, although the fever-induced pathogenesis and the subsequent epileptogenesis are not fully understood. In our cohort, the majority of patients with FS showed hippocampal sclerosis on MRI (79%), or focal cortical dysplasia (5%), or both (2%). Out of the 25 patients with history of febrile seizures operated, all of them had HS (associated to FCD in 24% of cases) as histopathological finding, except one patient with a diagnosis of Polymorphous low-grade neuroepithelial tumor of the young (PLNTY). The relationship between febrile seizures, hippocampal sclerosis and malformations of cortical development has been investigated in animal models and humans. It has been suggested that cortical malformations predisposes to febrile seizures, and focal recurrent seizures, leading to hippocampal sclerosis (Tassi et al., 2009). Another explanation is that HS and MCD share a common embryonic damage (Blümcke et al., 2002).

To our knowledge, the association between history of FS and PLNTY has not been previously reported. Given the recent discovery of this new entity of low-grade epilepsy-

associated neuro-epithelial tumor (LEAT) and the little number of cases described, this observation can help to define the clinical picture of the syndrome.

Data from video-EEG monitoring showed that TLE share a quite uniform electro-clinical picture, despite the different etiologies, and are in line with the current literature data (French, et al., 1993; Blair, 2012).

CHAPTER 4: Surgery and Histopathology in TLE

METHODS

Resective surgery was performed for strictly therapeutic reasons in 140 out of 255 patients (55%). The remaining 115 patients were either excluded from surgery after the diagnostic process for discordance of electro-clinical and radiological data, or patients refused surgery, some others are still awaiting for the intervention or others have been operated only recently with too brief follow up (less than 6 months).
Surgeries performed were standard anterior medial temporal lobectomy or cortectomy with the resection of the presumed seizure onset zone, including the eventual anatomic lesion. When electro-clinical data and MRI findings did not identify the epileptogenic zone with sufficient precision, intracerebral Stereo-EEG (SEEG) was performed to plan the resection.

Histopathological diagnosis was conducted according to international guidelines: hippocampal sclerosis was defined as segmental pyramidal cell loss, which can affect any sector of the cornu ammonis and classified in 3 subtypes, as specified in the consensus classification of the ILAE (Blümcke et al., 2013). Brain tumors were classified according to the 2007 WHO classification of tumors of the central nervous system (Louis et al., 2007). Focal cortical dysplasia was defined according to the consensus classification system of the ILAE (Blümcke et al., 2011). Cortical glial scar was diagnosed on the basis of gliosis in the cerebral parenchyma, subcortical white matter demyelination, and portions of normal temporal lobe structures with conserved neurons.

Negative histopathological findings, underlying cryptogenic TLE, were defined when no pathological lesions were found; unspecific gliosis or white matter neuron heterotopias were included in this category.

Surgical outcome was obtained according to Engel Classification at last follow-up, at least 6 months after surgery.

RESULTS

One hundred and forty patients completed the presurgical evaluation and received resective surgery in the temporal lobe.

Stereo-EEG was performed in 37 patients for a better definition of the seizure onset zone: in 20 patients MRI was negative, in the remaining 17 patients MRI showed MCDs (6 PNH, 6 PMG, 1 HS plus FCD), 3 patients had a scar and one tumor.

The results of the histopathological diagnosis are presented in Table 2.

Hippocampal sclerosis was the most frequent histopathological finding, associated to focal cortical dysplasia in the 30% of cases; the second most frequent etiology was low-grade tumors, and the third one was represented by FCD without HS.

A favorable surgery outcome (Engel Class I) was obtained in the 87% of all cases (n=123). Considering outcome in relation to different diagnosis, excellent outcome was observed for patients having tumors, scars or cavernous angiomas on pre-operative MRI; less favorable outcome (Engel Class III and IV) was found in patients with negative MRI, or with FCD.

DISCUSSION

Surgical and histopathological data from 140 patients with TLE have been reviewed. In the present multicentric study 26% of patients needed invasive intracerebral exploration with Stereo-EEG to define the epileptogenic zone. This data appears lower than a

previous american study in which the 35% of patients with TLE underwent intracranial EEG monitoring (Sperling et al., 1996), while it appears higher then a previous italian work on TLE in which 17% of patients received stereo-EEG (Tassi et al., 2009). As observed in a recent multicentric European survey on pediatric epilepsy surgery, invasive recordings were used in pediatric cases for both temporal and extra-temporal epilepsy surgery in the 20% of cases, with an increased proportion of stereo-EEG recordings in newer centers compared to older centers (Barba et al., 2019).

In the present study the absence of epileptogenic lesion on MRI represented the main reason for stereo-EEG recordings, in line with previously reported series. The remaining cases were patients with complex malformations of cortical development (MCD) on MRI. To note, in the totality of patients with periventricular nodular heterotopias (n=6) stereo-EEG was performed not only as a diagnostic tool for the identification of the seizure onset zone, but also as a treatment option through stereo-EEG guided thermocoagulations (THC). All patients had good seizure outcome after THC and did not proceed to resective surgery (Mirandola et al., 2017).

The most frequent histopathological diagnosis was hippocampal sclerosis, accounting for 32% of cases (22% pure HS, and 10% in association to MCD), followed by tumors, and then focal cortical dysplasia. These results are consistent with a recent European multicentric study on approximately 10.000 specimens obtained during surgery for drug-resistant focal epilepsy (Blümcke et al., 2017).

Although HS is still the main cause of temporal lobe epilepsy, and focal epilepsy in general, the proportion of patients with this disease is decreasing in comparison to the past years when it represented 70-80% of etiologies of TLE (Sperling et al., 1996; Wiebe et al., 2001). An explanation might be that since the discovery of the syndrome

characterized by temporal lobe epilepsy and HS on MRI (Berkovic et al., 1991) more and more patients received the diagnosis and sequentially performed the surgical treatment. Secondly, HS has been associated to a recurrent insult to the brain during childhood, such as febrile seizures, or prolonged seizures, therefore improvement in the management of this conditions can lead to reduction in the secondary development of HS.

It is to note that newly characterized histopathological entities have also been observed in our cohort, specifically 3 patients with diagnosis of polymorphous low-grade neuro-epithelial tumor of the young (PLNTY), in one case associated to FCD. PLNTY is a recently described variant of LEAT that exhibits infiltrative growth, histopathological variability with frequently prominent oligodendroglioma-like components, intense labeling for CD34, absence of 1P/19Q codeletion, a distinct DNA methylation signature and genetic alterations (Huse et al., 2017). In the 3 cases here reported, mean age at epilepsy onset was 17 years, with a short duration of epilepsy before surgery (2.6 years), in line with previously described cases. In 2 cases the MRI diagnosis was that of a generic low-grade tumor while in the third case focal cortical dysplasia was hypothesized. Although limited by the small number of patients, no clear differences in the electro-clinical features in comparison to the other etiologies have been observed.

Overall, surgical outcome was good, with 87% of patients belonging to Engel Class I, and 77% completely free from seizures (Engel Class Ia). This finding confirms that TLE is a surgically remediable syndrome with high chance of recovery from seizures. Comparing to series from the past with 55-70% of good post-surgical outcome (Sperling et al., 1996, Engel et al., 1999, Wiebe et al., 2001) an increment in favourable outcome has been observed. This might be related to an improvement in the selection of patients

eligible for surgery, thanks to a better knowledge of the syndrome and the advance in neuroimaging techniques.

As previously observed (Berkovic et al., 1995, Berg et al., 1998) a positive outcome is associated to the presence of mesial temporal sclerosis, a known etiology, and only focal seizures. In the present study no significant differences based on MRI diagnosis have been highlighted, only a trend of a less favourable outcome for patients with negative MRI or FCD. A recent work performed by one of the 4 Epilepsy Centres of the study ("Claudio Munari" Epilepsy Surgery Centre) underlined that independent negative prognostic factors for MRI-negative TLE were the presence of auditory aura and contralateral diffusion of IEDs on EEG, while the use of 18-FDG PET was a positive prognostic factor. Interestingly, no differences in seizure outcome in histologically negative or positive patients were observed, suggesting that if carefully selected, patients with MRI-negative TLE may be good candidates to resective surgery (Mariani et al., 2019).

CHAPTER 5: Structural MRI findings in TLE

METHODS

3T brain MRI (Philips or Siemens) was obtained for all patients. The standardized protocol performed at all Epilepsy Centres included the following anatomical sequences: diffusion weighted imaging with apparent diffusion coefficient mapping (DWI_ADC), 3D fluid attenuated inversion-recovery (Flair_3D), coronal Flair (Flair_COR), coronal T2-weighted imaging (T2_COR), coronal T1-weighted imaging phase-sensitive inversion recovery (T1_PSIR_COR), 3D T1‑weighted MRI (T1-3D).

Intravenous contrast was injected when necessary. For each Centre two experts in epilepsy surgery (epileptologist and neuro-radiologist) reviewed the images. In case of discordant results, images were reviewed until a consensus was found. Focal lesions, hippocampus atrophy, gyration anomalies, focal thickening of the cortex, blurring of the grey-white matter junction, areas of abnormal signal intensity in the cortex and white matter, and vessel anomalies were assessed.

Diagnostic power of 3T structural MRI was evaluated: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for pre-operative MRI.

RESULTS

All patients concluded the structural MRI protocol. In 22% of patients (n=56) no alterations compatible with epileptogenic lesion were found and were therefore considered as negative MRI. In the remaining 78% of cases different pathologies have been diagnosed on MRI as summarized in Table 2. Eighty-two patients had right-sided lesions, 112 left-sided and 8 bilateral. Hippocampal sclerosis was the most frequent

diagnosis, observed in more than one third of the cases, followed by low-grade tumors and then focal cortical dysplasia (associated or not with HS). Other MCD such as periventricular nodular heterotopia or polymicrogyria were less frequently diagnosed. Cavernous angioma was the only vascular malformation observed, accounting for 3% of the total causes of TLE.

Table 2: Pre-operative 3T MRI findings and histopathological diagnosis.

	MRI findings		**Histopathological findings**	
	Number of patients (Total 255)	**%**	**Number of surgical procedures (Total 140)**	**%**
Negative	56	22	31	22.1
Pure HS	82	32.2	32 (29 HS1, 1 HS2, 2 HS3)	22.8
HS + MCD	2	0.8	14 (9 HS1+FCD IIIa)	10
Tumor	42	16.5	26	18.7
Scar	6	2.3	5	3.6
Periventricular nodular heterotopia	6	2.3	-	
Focal cortical dysplasia	34	13.3	17	12.1
Polymicrogyria	5	2	2	1.4
Cavernous angiomas	8	3.1	6	4.3
Encephalitis	1	0.4	2	1.4
Other MCD	-		1	0.7
Perinatal ischemic damage	3	1.2		
Other	10	3.9		
Not available			4	2.9

HS: hippocampal sclerosis. MCD: malformations of cortical development.

Overall, pre-operative MRI sensitivity resulted high (96.2%) while specificity in detecting any cause of TLE, was relatively low (67.7%). PPV was 90.9% and NPV

84%. When considering single etiologies, MRI resulted very sensitive and specific in detecting hippocampal sclerosis and angiomas cavernous compared to other causes (Table 3).

Table 3: Sensitivity and specificity of pre-operative MRI for different etiologies.

	Sensitivity	Specificity
Hippocampal sclerosis	91%	93.3%
Tumors	84.6%	91.8%
Focal cortical dysplasia	58.8%	95.7%
Cavernomas	100%	99.2%

DISCUSSION

The use of a standardized structural MRI protocol in 255 patients with drug-resistant TLE revealed an epileptogenic lesion in the 78% of patients.

The most frequent diagnosis was hippocampal sclerosis, observed in 32% of cases, followed by low-grade epileptogenic tumors (16% of cases) and focal cortical dysplasia (13%). These findings are in line with the first three causes of TLE histologically diagnosed (Blümcke et al., 2017).

Sensitivity and specificity of the MRI was very high for the detection of HS and cavernomas in particular, while brain MRI was less sensitive in the diagnosis of FCD, which is not surprising. On MRI, FCD features are mainly related to a histopathological type (FCD type II) and include increased cortical thickness, blurring of the gray/white matter junction on T1-weighted and T2-weighted images (WI), an increased signal of subcortical white matter on T2 and FLAIR-T2WI, and a decreased signal on T1WI. The white matter signal alterations frequently taper towards the ventricle delineating the socalled “transmantle sign”. The combination of more than one radiological signs facilitates the diagnosis, although small and subtle FCD remain a challenge even for

expert neuroradiologist (Colombo et al., 2012). FCD type I are diagnosed histopathologically, often associated to another lesion such as HS or tumor, but are difficult to detect on MRI (Tassi et al, 2010).

In our cohort, 22% of patients had a negative-MRI. This percentage is slightly lower than the proportion of negative- MRI (30%) in TLE patients among tertiary care centers described in recent literature (Mulhofer et al., 2017), thus suggesting that a standardized structural MRI protocol can help the detection of small brain abnormalities. The recent consensus report from the ILAE Neuroimaging Task-force on the recommendations for the use of MRI in patients with epilepsy (Bernasconi et al., 2019) identified a set of 3 MRI sequences to optimize lesion detection (HARNESS-MRI: Harmonized Neuroimaging of Epilepsy Structural Sequences). The core of these sequences is represented by three-dimensional acquisitions, which allow optimal evaluation of brain anatomy and morphology through high resolution T1-3D, identification of hyperintensities related to gliosis and increased extracellular space through FLAIR-3D, and bi-dimensional high-in plane resolution sequence (T2-COR), which is perpendicular to the long axis of the hippocampus and is best suited to assess hippocampal internal structure.

The high proportion of positive MRI in our patients, who all performed a standardized MRI protocol including also HARMESS- MRI sequences, confirmed its efficacy in optimizing lesion detection. To our knowledge, this is the largest prospective study on TLE patients undergoing this neuroimaging protocol.

CHAPTER 6: Functional MRI findings in TLE

INTRODUCTION

Simultaneous EEG and fMRI (EEG-fMRI) recording is a functional neuroimaging technique, which reveals cerebral hemodynamic changes related to interictal epileptiform discharges (IEDs) visualized on scalp EEG. It combines the temporal resolution of the EEG with the spatial resolution of MRI, offering the opportunity to define epileptogenic foci, or complex epileptic networks (Krakov, 1999; Gotman, 2008). The analysis of continuous and simultaneous recording of the EEG during MRI scanning is possible by removing MRI-gradient artifacts and pulse-related (cardioballistogram) artifacts (Allen, 2000).

In the last 20 years several EEG-fMRI studies have been performed in patients with epilepsy to better understand specific epileptic networks in focal and generalized epilepsies.

The hemodynamic response to interictal epileptiform discharges (IEDs) could result in BOLD increments (or fMRI activations), reflecting spike-generating fields, or decrements (deactivations), which can be interpreted as areas of deafferentation, although the meaning of deactivation is still debated (Kobayashi, 2006).

In focal epilepsies, BOLD-signal changes have been found tightly coupled with the regions generating focal IEDs and concordant with intracerebral findings (Pittau, 2014). Conversely to intracerebral recordings that are targeted to few cerebral structures based on *a priori* hypothesis, the spatial resolution of EEG-fMRI is such to cover the whole brain, and can highlight the involvement of deep brain structures in seizure generation or propagation, and their relationships with the cortex (Kobayashi, 2006).

Patients with TLE have previously been studied with EEG-FMRI (Kobayashi, 2006; Salek-haddadi, 2006; Kobayashi, 2009; Fahoum, 2012, Pittau, 2012; Coan, 2016). Modifications of BOLD signal related to temporal IEDs were observed in 50-83% of studies, and often, but notably not in all cases, the fMRI response was localized in the temporal lobe, ipsilateral or/and contralateral to the IEDs on EEG, but also in extra-temporal cortical or subcortical structures. These findings are in line with the recent concept of "network epilepsy" applied to TLE which has overcome the traditional hypothesis that in focal epilepsy seizure activity originates from a specific and anatomically isolated focus (Berg et al., 2010; Avanzini et al., 2012).

Ideally, EEG-fMRI could represent a non-invasive way to identify the seizure onset zone as part of the presurgical evaluation, but its clinical use is still limited. Modifications of the BOLD signal in relation to IEDs do not distinguish between "irritative" zone, seizure onset zone, or propagation effect (Laufs, 2007).

The main difficulty of studies that focused on its clinical role in presurgical evaluation was the small number of temporal spikes or other IEDs recorded during the scan. Efforts have been made to overcome the issue, and a new method (Grouiller, 2011) has been applied in patients without visually detectable IEDs: haemodynamic changes can be revealed by correlating epilepsy-specific voltage maps, derived from EEG recorded during long term video-telemetry monitoring, with the intra-scan EEG.

A few retrospective EEG-fMRI studies on patients with TLE undergoing epilepsy surgery found that if the BOLD response was located in the area that was surgical removed (concordant fMRI response) the post surgical outcome was better than if the BOLD response was outside the surgical resection (discordant fMRI response). (Thornton et al., 2010; Coan et al., 2016).

Studies on group-analysis of EEG-fMRI data aimed to reveal a common network alteration in patients with TLE, compared to other focal epilepsies. BOLD activations related to IEDs were found in the ipsilateral mesial and neocortical temporal cortex, but also in the insula and the cerebellum, while deactivations were observed in structures belonging to the Default Mode Network (DMN) (Laufs et al., 2007, Fahoum et al., 2012). The DMN is a physiological cerebral network comprising brain areas preferentially active on functional imaging during conscious rest, including the precuneus and posterior cingulate, bilateral temporo-parietal and medial prefrontal cortices (Raichle et al., 2001).

Several evidences seem to confirm the hypothesis that TLE affects resting state networks (Cataldi et al., 2013). fMRI studies showed differences between patients with epilepsy and healthy controls in the spontaneous activity of specific nodes of these networks (Zhang et al., 2010). In mesial TLE in particular, a diffuse impairment in the functional connectivity in nodes of these networks have been observed (Liao et al., 2011; Fahoum et al., 2012). Because the activity of a network is dependent on the functional interaction of its nodes, even small changes in connectivity may cause dysfunction in global brain networks. Alterations in the major resting state networks including DMN, language, executive control, attention and the reward/emotion networks have been observed in patients with TLE compared to controls, suggesting that TLE modifies chronically the activity of brain networks that control basic functions such as cognition, attention and emotion (Cataldi et al., 2013).

Thirty-three patients with TLE undergoing EEG-fMRI as part of the presurgical work-up were consecutively enrolled at the University of Modena and Reggio Emilia -

Epilepsy Center. The main purpose was to verify the contribution of this non-invasive technique in the clinical environment.

Three sub-studies were performed:

A. EEG-fMRI spike-triggered single-subject analysis. fMRI changes guided by interictal (or ictal) epileptiform activity were searched for each patient and compared with the epileptogenic zone. We evaluated if the fMRI response was localized in the temporal lobe or if a more widespread activation or deactivation was present. We also investigated if the fMRI changes were observed in cerebral structures belonging to the major resting state networks.
B. EEG-fMRI spike-triggered group analysis. The objective was to identify the core network of TLE patients eligible for surgery.
C. Seed-based resting state functional connectivity analysis. The aim was to investigate changes in the functional connectivity between the amygdala and the hippocampus and the rest of the brain.

METHODS

Participants

Thirty-three patients concluded EEG-fMRI co-registration (14 males; mean age 36.5 $\pm$ 10.3 years).

For resting state between-group comparisons, 24 healthy controls (16 males; mean age 30 $\pm$ 8 years) with no history of neurological diseases took part in the fMRI study.

All subjects gave their written informed consent to take part in the study.

Resting-state Video-EEG-fMRI protocol

Scalp EEG was recorded by means of a 32 channels MRI-compatible EEG recording system (Micromed, Mogliano Veneto, Italy). Electrodes were placed according to conventional 10–20 locations and the reference was FCz. ECG was recorded from two chest electrodes. Before scanning, 10 min of out-of magnet EEG data were collected. Foam pads were used to help secure the EEG leads, minimize motion, and improve the comfort of the patient. Data were transmitted via an optic fiber cable from the high-input impedance amplifier (1024 kHz sampling rate) to a computer located outside the scanner room.

Patients were also constantly observed and recorded by a small camcorder positioned on the head coil inside the scanner pointing to the patient's face to obtain a split-screen video-EEG documentation during the fMRI recording (Chaudhary et al., 2010; Ruggieri et al., 2015). Video data were used to monitor behavioral sign of sleep and movements. Functional data were acquired using a Philips Achieva system at 3T and a gradient-echo echo-planar sequence from 30 axial contiguous slices (TR=2000 ms; in-plane matrix=80x80; voxel size: 3x3x4) over one to three 8 minutes sessions per participant (240 volumes) with continuous video-EEG recording. The mean duration of the recording was 24 minutes (range 8-32 minutes), depending on the number of acquired sessions. A high-resolution T1-weighted anatomical image was acquired for each participant to allow anatomical localization. The volume consisted of 170 sagittal slices (TR= 9.9 ms; TE= 4.6 ms; in plane matrix= 256x256; voxel size=1x1x1 mm).

EEG analysis

EEG acquisition was used to identify interictal (or ictal) epileptiform activity during the fMRI session. BrainQuick System Plus software (Micromed) was used for offline correction of the gradient artifacts (Allen et al., 2000) and filtering of the EEG signal. In

addition, the EEG data were exported in the .edf format and reviewed and analyzed by means of the BrainVision Analyzer 2.0 software (Brain Products, Munich, Germany). After removing the gradient and mean ballistocardiographic artifacts, according to previous published methods (Vaudano et al., 2014), an experienced electroencephalographer reviewed the preprocessed EEG recordings (LM) to identify interictal epileptiform abnormalities based on both spatial distribution and topography. When recognized, IEDs were marked as intervals. We classified patients as unilateral (right or left) in case of only one spike focus without contralateral spreading; bilateral in the case that both foci were active. In this latter condition, left and right IEDs were considered together in further analyses.

A) SINGLE-SUBJECT ANALYSIS

fMRI analysis

fMRI data analyses were performed using MATLAB version R2013a (The MathWorks Inc, Natick, Mass) and SPM12 (Wellcome Department of Imaging Neuroscience, London, UK). Pre-processing of functional volumes included slice timing correction, realignement to the first volume acquired and smoothing with a 6x6x8 mm FWHM Gaussian kernel. T1-weighted anatomic sequence was coregistered with the mean EPI for each subject.

The interictal (or ictal) epileptiform activity was implemented as regressor of interest in the single patient-first level analysis. In order to do so, patients' IEDs were treated as a single event regardless of their topography or location. Their onset was exported in .mat file that describes the exact timing (in seconds) of IEDs for fMRI time bin (TR=3/2sec). The resulting timing files served as onset for GLM convolved with the standard hemodynamic response function (HRF) and its temporal and special derivatives (TD,

DD). This procedure reflects the standard procedure generally adopted in previous works from our group and others (Meletti et al., 2016; Siniatchkin et al., 2010; Moeller et al., 2013).

The movement parameters estimated during the realignment were included as nuisance regressors.

For each patient, specific contrasts were settled up to test the BOLD effect of IEDs with respect to the resting EEG background.

RESULTS

Sixty-eight patients enrolled at the 4 centres concluded EEG-fMRI co-registration protocol. Here we present the results from 33 patients recorded at University of Modena and Reggio Emilia- Epilepsy Centre.

The mean duration of the recording was 24 minutes (range 10-40 minutes). No seizures were recorded. In 25 patients (75%) epileptiform activities resembling patient's out-of-scan typical abnormalities were identified. In the remaining 8 patients no clear epileptiform discharges were found therefore the fMRI analysis could not be performed. Interictal epileptiform discharges (IEDs) found during intra-scan EEG are described in Table 4.

In all patients spike-triggered fMRI changes were obtained.

To address the question if EEG-fMRI had a localizing value, we looked for the overlap of BOLD clusters (positive or negative) and the Epileptogenic Zone (EZ) for each subject. In 14 patients (56% of cases) fMRI changes were observed in the temporal lobe ipsilateral to IEDs, although only in 2 patients (8%) a clear cluster overlapping the EZ identified by anatomo-electro-clinical correlations, and confirmed by post surgical outcome, was observed. In this first group of patients BOLD increments or decrements

were observed also in extratemporal structures. We will refer to this group as “Temporal Plus” group.

In the remaining 11 patients (44%) fMRI responses guided by temporal IEDs were localized outside the temporal lobes. We called this second group “Extra-temporal” group.

Differences in clinical, radiological, etiological and surgery outcome data were evaluated. The only statistically significant difference ($p=0.0172$) was observed in the etiology: hippocampal sclerosis diagnosed on MRI and confirmed histopathologically, was more frequently observed in the “Extra-temporal” group. No differences in surgery outcome were present between the 2 groups.

The involvement of a major resting state network was investigated as second step of fMRI data interpretation. In 11 patients DMN BOLD changes (either positive or negative) were observed; the sensory-motor network was involved in 7 patients, the salience network in 5 patients, the central executive network in 4 patients, and the visual network in 2 patients (Table 4).

Table 4: EEG- fMRI recordings data from 33 patients with TLE.

Pt	Type of EEG abnormalities	fMRI results localization	Resting states Networks	Structural MRI Diagnosis	Surgery/ histopathology
207	SW (R)	Temporal +	Bilat postDMN+	FCD	no
208	SW (R)	Extra-temporal	L visual+	HS	Yes/HS1
210	SW (bilateral)/ Slow (L)	Temporal +	R DMN+, R sensory +	HS	Yes/gliosis
211	SW (bilateral L>R)	Temporal +	Bilat perisylvian+	Tumor	no
213	SW (L with diffusion to R)	Temporal +	Bilat sensory +, L visual +	FCD	no
214	SW (L), Slow (L)	Extra-temporal	Bilat perisylvian+	HS	Yes/gliosis
215	SW, Slow (bilateral R>L)	Temporal +	Bilat postDMN+	HS	Yes/HS1
216	none			Perinatal Insult	no
217	SW (L)	Extra-temporal	Bilat perisylvian+	HS	no
218	SW (R), Slow (R)	Extra-temporal	L DMN+	HS	Yes/HS1
219	SW (L), Slow (bilat)	Temporal +	Bilat perisylvian-, Bilat postDMN-	Negative	no
220	SW (bilateral)	Extra-temporal	Bilat sensory-motor+	HS	no
221	None			HS	no
222	None			Negative	no
223	SW (bilateral)	Extra-temporal	R post DMN+	HS	no
224	none			Negative	no
225	SW , Slow (L with diffusion to R)	Temporal +	R sensory+, bilat postDMN+	Tumor	Yes/ ganglioglioma
226	Slow (bilateral L>R)	Temporal +	Bilat sensory-motor+, bilat persylvian +	Negative	no
227	SW , Slow (L with diffusion to R)	Temporal +	Bilat ant-mesDMN-	Tumor	Yes/ ganglioglioma
228	SW (R)	Extra-temporal	Bilat sensory-motor+	HS	Yes/HS+FCDIIIa
229	none			HS	Yes/FCD Ib
230	none			Negative	no
231	Slow (bilateral L>R)	Extra-temporal	Bilat mesDMN-, bilat executive+	Altro	no
232	Slow (R)	Temporal +	Post DMN-, perisylvian+	Tumor	Yes/DNET
233	SW (L)	Temporal +		Negative	no
234	SW (R)	Temporal +	Bilat postDMN+, L executive+	HS	Yes/HS1
235	none			Scar	no
236	none			FCD	no
237	Slow (bilat)	Extra-temporal	L executive+	Cavernoma	Yes/cavernoma
238	Slow (R)	Extra-temporal	postDMN+, bilat executive+	HS	no
239	Slow (bilat)	Temporal +		FCD	no
240	SW (R)	Temporal +		Tumor	Yes/PLNTY
241	SW , Slow (L with diffusion to R)	Extra-temporal	bilat sensory-motor+	Cavernoma	yes/

SW: spike-and-wave, slow:slow wave, R:right, L:left, bilat:bilateral, DMN:default mode network, +: BOLD signal increments, -: BOLD signal decrements, FCD: focal cortical dysplasia.HS:hippocampal sclerosis. DNET: dysembryoplastic neuroepithelial tumor. PLNTY: Polymorphous low-grade neuroepithelial tumor of the young.

B) GROUP ANALYSIS

fMRI analysis

Using the parameter estimates obtained by single-subject analyses, we performed two second level (group) random-effect analyses:

-one taking into account all the patients as a uniform group

-one dividing the patients in two groups: Hippocampal Sclerosis vs No Hippocampal Sclerosis.

To this end, patients' functional volumes were slice-time corrected, realigned and normalized to the MNI (Montreal Neurologic Institute) template implemented in SPM12. Finally functional images were spatially smoothed (6x6x8 mm FWHM Gaussian kernel).

Note that, for the EEG-fMRI spike-triggered group analysis and for the seed-based resting state analysis, functional volumes of right TLE patients were right-left flipped so the ipsilateral hemisphere was on the left in all cases. This was done using FSL (fslswapdim tool) before the above-mentioned preprocessing and normalization, in order to ensure a homogeneous group, with all patients having the seizure focus on the same side.

The threshold for statistical significance was set at $p<0.001$ (uncorrected) and cluster extent of 13 voxels.

RESULTS

The group analysis including all patients (n=33) did not reveal common fMRI BOLD changes in relations to interictal EEG abnormalities, considering both spike-and-waves, and slow-waves.

Since the group was heterogeneous we decided to perform the analysis for a subgroup of patients sharing a common etiology, hippocampal sclerosis, which was the most frequent one. The group analysis of these patients (n=12), guided by both EEG abnormalities (spike-and-waves and slow-waves) showed an increment of BOLD signal in the precuneus (Figure 1).

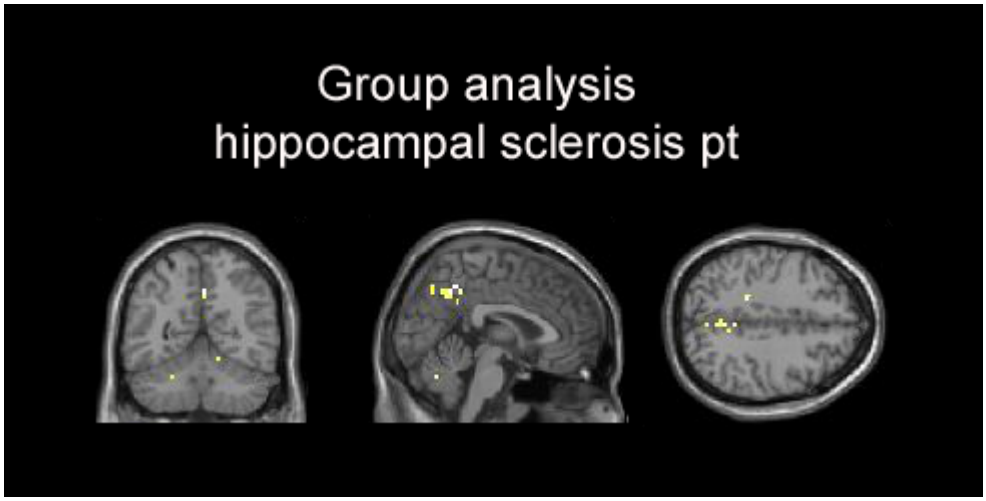

Figure 1: Second-level group analysis from 12 patients with hippocampal sclerosis. Coronal, sagittal and axial images showing increment of BOLD signal in the precuneus. $p < 0{,}005$

C) SEED-BASED FUNCTIONAL CONNECTIVITY ANALYSIS

fMRI analysis

fMRI data analysis was performed using MATLAB version R2013a (The MathWorks Inc, Natick, Mass) and SPM12 (Wellcome Department of Imaging Neuroscience, London, UK). Functional volumes of each participant were slice time corrected, realigned to the first volume acquired and normalized to the MNI (Montreal Neurologic Institute) template implemented in SPM12. A temporal filter (0.01-0.08 Hz) was applied using Resting-State fMRI Data Analysis Toolkit (REST) (Song et al., 2011) to reduce

low frequency drifts and high frequency physiological noise. Finally, functional data were smoothed with a 6x6x8 mm FWHM Gaussian kernel.

Left-right hippocampus and amygdala belonging to the AAL Atlas (Tzourio-Mazoyer et al., 2002) were used as Region of Interest (ROI). The BOLD signal time course was extracted from left and right seeds of each patient and healthy control by means of marsbar (http://marsbar.sourceforge.net/). Four separate first level (single subject) regression analyses were performed for each subject, using the BOLD signal time course of the seed regions as predictor of interest. The six head-motion parameters (translations and rotations) and the time courses representing mean signal fluctuations in gray matter, white matter and cerebrospinal fluid were entered as confounds. Single patient contrast images were generated for each seed and were then included in a second-level full factorial model with a 2 x 2 design (group [patients vs healthy controls] x ROI [left and right amygdala/hippocampus]). Age and gender were included as covariates.
A double statistical threshold (voxel-wise $p < 0.001$ and spatial extent) was adopted to achieve a combined significance, corrected for multiple comparisons, of $\alpha < 0.05$, as computed by 3dClustSim AFNI routine, using the "-acf" option (https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html).
Regression analyses were performed to examine correlation between hippocampus and amygdala functional connectivity and disease duration (years).

RESULTS

Seed-based functional connectivity results are shown in Table 5.
Right and left amygdala used as Region of Interest (ROI) separately showed no significant changes in the functional connectivity compared to controls, whereas considering both sides together revealed alterations. In particular increased functional

connectivity was observed in patients with TLE compared to healthy subjects between amygdala and right middle and inferior frontal cortex, right inferior parietal lobule/supramarginal gyrus, and left thalamus. Decreased functional connectivity was observed between amygdala and the cerebellum bilaterally.

Functional volumes of right TLE patients were right-left flipped so the hemisphere ipsilateral to epilepsy was on the left in all cases. The left hippocampus represents therefore the epileptic side, while the right hippocampus the non-epileptic one. Increased functional connectivity compared to controls was found between the epileptic hippocampus and the ipsilateral temporal neocortex (middle temporal gyrus), bilateral middle frontal gyri and bilateral supramarginal gyri; decreased functional connectivity was instead observed between the left hippocampus and the mesial regions of the DMN (precuneus, superior frontal gyrus, anterior cingulate cortex), the contralateral temporal regions (parahippocampus and fusiform gyrus) and the cerebellum (Figure 2).

The right hippocampus (the non-epileptic side) appeared less functionally connected compared to controls with the contralateral anterior cingulate cortex, inferior frontal gyrus and posterior cingulate cortex.

A hip conjunction was performed between left and right hippocampus, confirming that only the epileptic hippocampus was less connected with the precuneus.

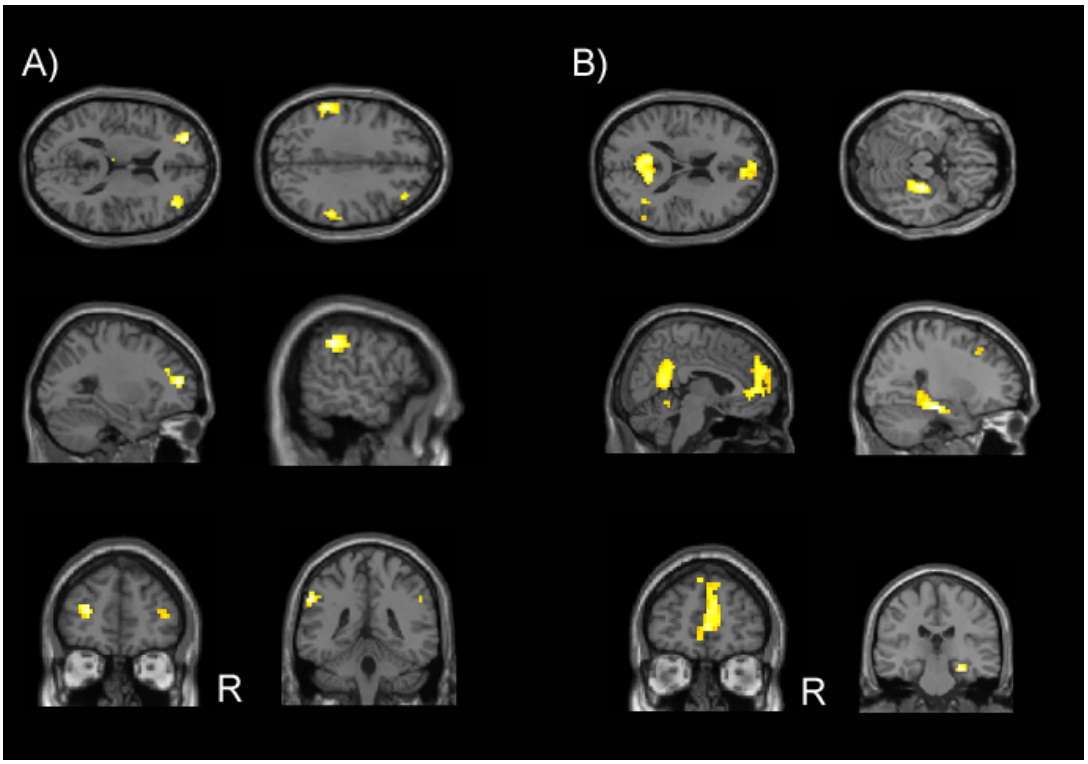

Figure 2: Axial, sagittal and coronal images showing areas of increased functional connectivity (A) and decreased functional connectivity (B) of the left hippocampus in TLE patients respect to controls. P<0,005

Table 5: Seed-based functional connectivity data

Region of Interest (ROI)	**Cluster**	**Highest t level Description**	**Peak MNI coordinate (x, y, z)**	**Peak T**
R+L amygdala (pt vs ctr)	1	L thalamus	-12, 31, 6	5,38
	2	Corpus callosum	-3, -22, 18	4,62
	3	R middle frontal gyrus	42, 41, 22	5,7
	4	R inferior frontal operculum	51, 5, 18	5,15
	5	R inferior parietal lobule	48, -40, 50	4,31
R+L amygdala (ctr vs pt)	1	L cerebellum	-6, -43, -6	6,53
L hippocampus (pt vs ctr)	1	L middle temporal gyrus	-48, -61, 2	3,98
	2	R middle frontal gyrus	39, 38, 30	4,71
	3	L middle frontal gyrus	-27, 47, 14	5,57
	4	Corpus callosum	-3, -22, 18	5,21
	5	R supramarginal gyrus	54, -34, 34	4,4
	6	L supramarginal gyrus	-57, -40, 34	5,36
L hippocampus (ctr vs pt)	1	R parahippocampal gyrus	27, -25, 18	5,87
	2	L cerebellum	-3, -43, -10	4,52
	3	R superior frontal mesial gyrus	3, 50, 6	5,26
	4	R angular gyrus	42, -58, 26	4,67
	5	R middle frontal gyrus	24, 29, 46	3,98
R hippocampus (ctr vs pt)	1	L inferior frontal gyrus	-36, 17, -18	4,4
	2	L anterior cingulate cortex	0, 41, -2	4,38
	3	L posterior cingulate cortex	-15, -61, 10	3,96

R:right, L:left, ctr: controls, pt:patients

DISCUSSION

Thirty-three patients with TLE eligible for surgery underwent simultaneous EEG-fMRI co-registration. Interictal epileptiform discharges were recorded in the 75% of the patients (n=25) therefore fMRI analysis could not be performed in all cases. In all patients with intrascan IEDs recording an fMRI response was obtained.

These results are in line with previous EEG-fMRI studies, confirming that a major limitation for the use of the technique in the presurgical setting is the absence of IEDs recording during EEG-fMRI (Coan et al., 2016), and in particular for mesial TLE, which often generates infrequent scalp-detectable IEDs (Kowalczyk et al., 2020).

When IEDs were recorded an fMRI response was obtained but in our series very rarely a BOLD cluster overlapped the Epileptogenic Zone (a clear overlap was observed in only 2 patients), suggesting that EEG-fMRI in patients with TLE is not a useful tool for localization purposes. In agreement with a recent retrospective review investigating the clinical impact of EEG-fMRI in the presurgical setting (Kowalczyk et al., 2020), we believe that in patients with TLE EEG-fMRI is not essential for the surgical decision. When a clear epileptogenic lesion is detected on structural MRI, which matches the epileptogenic focus defined by the video-EEG monitoring data, EEG-fMRI does not add information for the surgical planning. This was the case for the 82% (n=27) of our cohort. In the remaining 6 patients with negative MRI, in 3 patients no IEDs were recorded during EEG-fMRI, and in other 3 cases the fMRI response was represented by widespread changes of BOLD signal, not informative to localize the EZ.

Despite this observation, a constant fMRI response was detected for every patient in relation to the epileptiform discharges recorded from temporal derivations on scalp EEG. To better understand our findings we needed to move from a *focus* view to a *network* view, which is what a functional technique such as EEG-fMRI allows to do, highlighting a patient's specific epileptic network. Advancement in functional neuroimaging lead to the observation that TLE is not only a disease of certain brain structures involved in seizures onset ("the focus") but it affects chronically the activity of brain networks that control basic functions such as attention, emotion and cognition (Cataldi et al., 2013).

It was interesting to note that in our cohort fMRI changes occurred in the temporal lobe in the 56% of cases (n=14), while in the remaining 44% of patients, only extra-temporal BOLD activations or deactivations were observed. When comparing the two groups we noticed that the majority of patients with HS belonged to the extra-temporal group and that this difference was statistically significant (p=0.0172). In other words, for a patient with TLE due to HS, the spike-triggered fMRI response is more likely to be outside the temporal lobe. On one hand, being HS a well known surgically remediable syndrome, and the most frequent cause of TLE, these results corroborate the hypothesis that EEG-fMRI is not essential for focus localization in the presurgical planning. On the other hand, our findings suggest that TLE is a network disease that involves brain structures beyond the temporal lobes, which might explain cognitive and psychiatric co-morbidities.

We observed that IEDs-triggered fMRI changes involved parts or the whole of few major resting state networks, at single subject level. In particular we observed changes in the Default Mode Network (DMN) (n=11), in the sensorimotor network (n=7), in the salience network (n=5), the executive control network (n=4), and the visual network (n=2). Patients could have more than one resting state network involved.

Alterations in DMN (posterior cingulate cortex, medial prefrontal cortex and lateral parietal cortex) have already been reported in TLE patients, both through EEG-fMRI group analysis (Laufs et al., 2007; Fahoum et al., 2012) and resting state functional connectivity analysis (Liao et al., 2011; Zhang et al., 2010; Pittau et al., 2012; Cataldi et al., 2013). How the interictal epileptic activity alters the network is still unknown, although it has been suggested that DMN dysfunction might have a role in cognitive impairment (such as memory deficits) commonly observed in TLE patients.

The salience network constitutes the dorsal anterior cingulate cortex, bilateral insula and pre-supplementary motor area, and its dysfunction affects the functioning of other networks; it is also indispensable during the rapid change of behavior (Smitha et al., 2017). The executive network includes the dorsolateral prefrontal cortex and posterior parietal cortex, and it gets activated during fMRI tasks involving executive functions (Smitha et al., 2017).

The finding of spike-triggered BOLD changes in areas belonging to these higher functions networks might represent a neuroimaging sign that temporal IEDs affect cognition in TLE patients. A correlation with neurocognitive testing will be necessary to confirm this hypothesis.

Another interesting finding was the observation of BOLD changes in the sensorimotor network, which is not perhaps expected in patients who do not usually present sensory or motor impairments. Nevertheless, this result is consistent with extra-temporal structural abnormalities found in patients with mesial TLE, which include reduction in cortical thickness of the precentral and paracentral gyri (Whelan et al., 2018).

Finally, BOLD clusters in the visual network have been observed in 2 patients: visual perception has been found unaffected in TLE patients from a clinical point of view, but fMRI studies have shown altered functional connectivity between the hippocampus and the visual cortex, although the meaning of the findings is not fully understood (Tong et al., 2019).

Due to the limited number of the patients we could not evaluate differences in terms of clinical features between the subgroup of patients with diverse resting state networks alterations, but it would be interesting in the future to investigate them, and to add this functional neuroimaging marker to the global picture of the patient.

With a second-level group analysis we aimed to look for common spike-triggered functional alterations in patients with TLE eligible for surgery. No such common epileptic networks were found for the 33 patients of the present study. The most likely explanation is that the group was heterogeneous in terms of etiology, duration of epilepsy, drug-therapies, and cognitive comorbidities. The results at single-subject level confirmed that diverse epileptic networks were found in TLE patients, suggesting that TLE is a disease with multiple phenotypes.

We therefore look for a homogeneous subgroup of patients, at least for the underlying cause, which was hippocampal sclerosis. In this second analysis a result was obtained, and it was represented by increment of BOLD signal in the precuneus bilaterally.

This cerebral structure is believed to have such a central role within the DMN that it is considered as a network itself. It has been suggested that it assists in many behavioural functions, and that is vital for autobiographical memory retrieval, emotional stimulus processing and reward outcome monitoring (Smitha et al., 2017).

Our data show that temporal IEDs in patients with TLE due to hippocampal sclerosis might interfere with the activity of the precuneus, representing a possible explanation of memory and emotion impairments found in patients with this disease.

The cognitive impact of epileptic interictal activity can be investigated also through the study of human resting state functional connectivity. Brain functions arise in fact by the interaction of widespread networks, named the Intrinsic Connectivity Networks (ICNs) that support cognitive processes such as language and memory (Shamshiri et al., 2019).

To investigate alterations in functional connectivity of patients with TLE we performed a seed-based functional connectivity analysis, using amygdala and hippocampus of both sides as seeds.

In the present study the analysis was conducted after a right-left flipping step to have on the left the ipsilateral (to TLE) side and on the right the contralateral one. This step allowed the inclusion of a bigger number of patients (n=33).

As previously observed (Laufs et al., 2007; Pittau et al., 2012, Burianova et al., 2017, Liao et al., 2011; Zhang et al., 2010) as a first result, altered functional connectivity (FC) was found between amygdala and hippocampus, and cortical regions belonging to the DMN, confirming its central role in this disease. In the majority of the studies a reduction in FC was observed within the DMN in TLE patients compared to healthy controls, while in the present study both decreased and increased FC were observed. In particular the precuneus/PPC and mesial prefrontal cortex appeared less functionally connected to the left hippocampus (or the epileptic one), while the dorsolateral regions, both frontal and parietal, showed an increased FC with the left hippocampus. Hyperconnectivity between seeds of a network might play a compensatory role for the loss of function of other region within the same network (Cataldi et al., 2013) and our results are in line with this hypothesis. DMN abnormalities in patients with mesial TLE have also been observed through the combination of two techniques, fMRI and diffusion tensor imaging (DTI) fiber tractography, which is a direct way to depict the structural connectivity of brain networks. In a neuroimaging study on 20 patients with TLE due to bilateral HS (Liao et al., 2011) the combination of the two techniques (fMRI and DTI) revealed decrement in both structural and functional connectivity between the PPC/precuneus and mesial temporal structures.

Secondly, a decreased connectivity between amygdala and epileptic hippocampus with the vermis of the cerebellum was found. Only one previous study on functional connectivity reported decreased FC between right hippocampus and lateral cerebellum (Pittau et al., 2012). The role of the cerebellum in epilepsy is debated, but there are

increasing evidences that it may act as a modulator of epilepsy processes and a potential treatment target (Kros et al., 2015; Wong et al., 2015). A recent neuroimaging study of structural covariance mapping demonstrated a change in the grey matter volume (GMV) of the cerebellar vermis in patients with TLE due to hippocampal sclerosis. In particular, a significant negative covariance between anterior vermis and mesial temporal structures was revealed in patients with HS, more prominent for left-sided TLE (Marciàn et al., 2020). Our findings support the hypothesis of the existence of a structural and functional temporal-cerebellum network in patients with TLE. We observed a decreased functional connectivity between the vermis and the left hippocampus (the epileptic one), while no alterations were found for the right hippocampus (the non epileptic one) and the cerebellum, suggesting that the network is ipsilateral to the epileptic focus.

Other differences between the functional connectivity compared to healthy controls were observed when considering the epileptic hippocampus or the non-epileptic one as seed for the analysis. The anterior areas of the DMN appeared less functionally connected with both hippocampi, while the posterior regions of DMN (the precuneus) were functionally less connected predominantly with the left hippocampus.

In other words the precuneus had a decreased functional connectivity with the epileptic hippocampus, but not with the contralateral, suggesting again its vital role in the epileptic network.

In conclusion, functional MRI provided evidence both at single subject and at group level that temporal lobe epilepsy is a disorder of brain structures extending well beyond the temporal lobes. Pathological networks, involving brain areas belonging to the Intrinsic Connectivity Networks, have been highlighted in relationship with interictal epileptiform activity by means of simultaneous EEG-fMRI. Although future studies are

needed to corroborate the data and correlate with cognitive testing, these findings might represent a radiological marker of TLE and could be applied to the clinical setting.

Nowadays surgical planning is mainly focused on the identification of the seizure onset zone, which need to be surgical removed to reach seizure freedom. In the era of advanced neuroimaging techniques that discover networks epilepsies, a prospective different surgical treatment is conceivable, perhaps through a less-invasive way of disconnecting fibres that “turn-off” the pathological network, instead of removing cerebral structures.

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