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Running Head: FUNCTIONAL CONNECTIVITY IN EPILEPSY

Title: Altered functional connectivity in mesial temporal lobe epilepsy

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Abstract

Growing evidence of altered functional connectivity suggests that mesial temporal lobe epilepsy (mTLE) alters not only hippocampal networks, but also a number of resting state networks. These highly coherent, yet functionally distinct brain circuits interact dynamically with each other in order to mediate consciousness, memory, and attention. However, little is currently known about the modulation of these networks by epileptiform activity, such as interictal spikes and seizures. The objective of the study was to use simultaneous EEG-fMRI to investigate functional connectivity in three resting state networks: default mode network (DMN), salience network (SN), and dorsal attentional network (DAN) in patients with mTLE compared to a healthy cohort, and in relation to the onset of interictal spikes and the period immediately prior to the spikes. Compared to the healthy participants, mTLE patients showed significant alterations in functional connectivity of all three resting state networks, generally characterized by dysconnectivity to prefrontal areas and increased connectivity to subcortical and posterior areas. Critically, prior to the onset of interictal spikes, mTLE patients showed dysconnectivity to the DMN and decreased synchronization within the SN and DAN, demonstrating alterations in functional coherence that may be responsible for the generation of epileptiform activity. Our findings demonstrate mTLE-related alterations of connectivity during the resting state as well as in relation to the onset of interictal spikes. These functional changes may underlie epilepsy-related cognitive abnormalities, because higher cognitive functions, such as memory or attention, rely heavily on the coordinated activity of all three resting state networks.

Keywords: mesial temporal lobe epilepsy; default-mode network; dorsal attentional network; salience network; functional connectivity

Altered functional connectivity in mesial temporal lobe epilepsy

In mesial temporal lobe epilepsy (mTLE), the most common form of focal epilepsy, a large-scale hippocampal network mediates the generation of interictal spikes and seizures (Faizo *et al.*, 2014; Hufnagel *et al.*, 2000; Janszky *et al.*, 2001). Recent evidence suggests that mTLE also alters activity in a number of resting state networks (RSNs). These highly coherent and functionally distinct brain circuits (Biswal *et al.*, 1995) interact dynamically with each other to mediate sensorimotor and cognitive functions, including consciousness, memory, or attention (Buckner *et al.*, 2008; Clemens *et al.*, 2011; van den Heuvel & Hulshoff Pol., 2010). Disruptions of RSNs have been linked to cognitive decline (Andrews-Hanna *et al.*, 2014; Sambataro *et al.*, 2010), depression (Sheline *et al.*, 2010), schizophrenia (Manoliu *et al.*, 2014), and Alzheimer's disease (Zhou *et al.*, 2010). Moreover, growing evidence of altered resting state connectivity in temporal lobe epilepsy (Liao *et al.*, 2010; Yuan *et al.*, 2013; Zhang *et al.*, 2010) raises the possibility of a role in epilepsy-related cognitive abnormalities, given the heavy reliance of higher cognitive functions, such as memory retrieval or language production, on the coordinated activity of RSNs.

In mTLE, alterations in RSN function have been studied most extensively in the default mode network (DMN), which comprises the posterior cingulate cortex (PCC), precuneus, medial prefrontal cortex, and its key structure: the hippocampus (Greicius *et al.*, 2003; Raichle *et al.*, 2001). In mTLE, studies show decreased connectivity between the hippocampus and the other DMN nodes at rest (Pittau *et al.*, 2012; Zhang *et al.*, 2010) and during interictal discharges, as measured by simultaneous EEG-fMRI (Fahoum *et al.*, 2012; Kobayashi *et al.*, 2006; Laufs *et al.*, 2007). Some argue that such alterations in DMN connectivity may underlie epilepsy-related deficits in declarative memory (Braakman *et al.*, 2012; Lin *et al.*, 2012), as the DMN is known to functionally subserve internally-driven cognitive operations, such as self-referential, language, and memory processes (Buckner *et*

al., 2008; Burianová *et al.*, 2010). Importantly, however, this network also plays a critical role in cognitive processes that are engaged during stimulus-directed tasks, as it dynamically interacts with two other large-scale functional networks: the dorsal attentional network (DAN) and the salience network (SN; Chen *et al.*, 2013; Elton & Gao, 2015; Vatansever *et al.*, 2015). In other words, we argue that during higher-level cognitive processing, aberrant hippocampal activity in mTLE patients would be associated with changes in functional connectivity not only in the DMN, but also in networks that dynamically interact with the DMN.

The DAN, comprising the intraparietal sulcus (IPS) and frontal eye fields (FEF), subserves top-down voluntary attention and visual working memory, whereas the SN, comprising the dorsal anterior cingulate and fronto-insular cortices, mediates the detection of behaviourally relevant stimuli and coordination of attentional resources towards such stimuli (Andrews-Hanna *et al.*, 2014; Chen *et al.*, 2013; Corbetta & Shulman, 2002; Fox *et al.*, 2005; Seeley *et al.*, 2007; Spreng *et al.*, 2013; Sridharan *et al.*, 2008). Albeit scarce, the available evidence demonstrates significantly decreased functional connectivity of the DAN in participants with mTLE during top-down attentional tasks (Liao *et al.*, 2010; Zhang *et al.*, 2009) and a correlation between poor performance on the tasks and severity of the connectivity alteration (Zheng *et al.*, 2012). The results of these studies suggest that the impairment in DAN connectivity might underlie attentional deficits in mTLE patients (Fleck *et al.*, 2002; Stella & Maciel, 2003); however, attentional dysfunction in mTLE has also been linked to disruptions in salience network connectivity.

To date, only one study has examined the intrinsic activity of the SN in epilepsy, showing a significant relationship between the SN and conscious awareness in childhood absence epilepsy (Luo *et al.*, 2014). It is speculated that atypical connectivity in the SN could

result in interruption of salient information processing, impairing salience detection and attentional processing (Uddin, 2014).

Despite accumulating evidence that deficits in higher-order cognitive functions, which frequently accompany mTLE, may be significantly related to disruptions of functional connectivity in large-scale RSNs, little is currently known about the modulation of these networks by epileptiform activity, such as interictal spikes and seizures. Although the relation between interictal spikes and seizures is not fully understood (Avoli *et al.*, 2006), evidence suggests that those neural structures that are involved in generating interictal spikes might be reliable estimators of the network or networks that generate seizures (Hufnagel *et al.*, 2000; Marsh *et al.*, 2010). Recent EEG-fMRI findings have demonstrated significant alterations that occur immediately *prior to* the appearance of interictal spikes, both in hemodynamic response (Jacobs *et al.*, 2009; Rathakrishnan *et al.*, 2010) and functional connectivity (Faizo *et al.*, 2014). The objective of the study was to use simultaneous EEG-fMRI, which provides highly spatially resolved three-dimensional maps of brain activity (fMRI) associated with interictal electrical discharges (EEG), to investigate functional connectivity in three resting state networks: DMN, SN, and DAN in participants with mTLE compared to a healthy cohort, and, to examine the relationship between changes in connectivity and the onset of interictal spikes.

Methods

Participants.

Fifteen patients with the diagnosis of mTLE (9 females, mean age: 38 years; 6 males, mean age: 42 years; 10 left and 5 right lateralized) and 15 age- and gender-matched healthy controls participated in this study. All patients were recruited from the Royal Brisbane and Women's Hospital Epilepsy clinic where they underwent comprehensive clinical assessment and the diagnosis of mTLE was based on the following: (i) seizure semiology consistent with

mTLE, (ii) interictal spikes confirmed during in-patient video-EEG monitoring performed within the last year, and (iii) MRI scan consistent with a temporal lobe focus (no lesion or ipsilateral hippocampal sclerosis). Patient exclusion criteria included absence of interictal spikes during monitoring, recurrent unprovoked seizures, and the presence of metal implants. Patients' clinical details and spike distributions are summarized in **Table 1**. Healthy controls were screened for current and previous brain injury, neurological and psychiatric disorders. All participants gave signed informed consent approved by the Human Research Ethics Committee (HREC) at the Royal Brisbane Women's Hospital (RBWH) and the Medical Research Ethics Committee of the University of Queensland.

[Insert Table 1 here]

Study procedure.

An MRI compatible 64-channel electrode cap, to record EEG from the patients, was positioned on patients' heads according to the international 10:20 system and prepared with a conductive non-abrasive gel (chloride 10%). All electrodes, including the ground (AFz) and reference electrodes (FCz), had an impedance below 5 kilo-ohms. One additional electrode recorded an electrocardiogram from the chest. Patients then underwent a 40-minute resting state, simultaneous EEG-fMRI recording in a 3T Magnetom Trio scanner (Siemens, Erlangen, Germany) and were instructed to remain still, awake, and relaxed with their eyes closed. The control participants underwent only resting state fMRI without the EEG recording.

EEG Data Acquisition & Preprocessing.

EEG data were recorded continuously during fMRI acquisition using Brain Vision recorder software version 1.20.0001 (Brainproducts Co., Munich, Germany). EEG preprocessing was conducted off-line using EEGLAB software (Delorme and Makeig, 2004). Gradient and ballistographic artefacts were removed with the Artefact Slice Template

Removal (FASTR) algorithm implemented in EEGLAB. Residual artefacts were removed using Independent Component Analysis (ICA), following which a neurologist reviewed the EEG recordings to identify interictal spikes.

fMRI Data Acquisition and Preprocessing.

A twelve-channel head coil was used to acquire the images and head pads were used to minimize head movements during the scan. A high-resolution T1-weighted MP-RAGE sequence was acquired with the following parameters: 192 slices, repetition time (TR) = 1900 ms, echo time (TE) = 2.13 ms, flip angle = 9° , matrix = 192 x 256 x 256, voxel size = 0.9 mm³. Images sensitised to BOLD contrast were acquired with a single-shot gradient-echo planar image sequence, using the following parameters: 36 slices, TR = 2.5 sec, TE = 30 ms, flip angle = 90° , field of view (FOV) = 210 mm, matrix = 64 x 64, voxel size = 3.3 mm³. EEG-fMRI data were collected in six runs, each lasting 5:05 minutes.

fMRI preprocessing was conducted using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) running on MATLAB. Functional images were slice-time corrected, realigned, and resliced using a 6-parameter rigid body spatial transformation (Friston *et al.*, 1995). The structural scan was coregistered to the mean functional image for each participant and normalized to the MNI template. Spatial smoothing was conducted on all normalized images using an 8mm FWHM Gaussian kernel.

fMRI Analysis.

We aimed to (i) compare functional connectivity, during rest, in DMN, SN, and DAN between the patients and healthy controls, and (ii) compare functional connectivity in DMN, SN, and DAN between the pre-spike and spike epochs *within* the patient group. For this purpose, we defined three 10-second epochs according to their relation to interictal spike onset: spike (0–10s), pre-spike (-10 to 0 s), and rest (-20 to -10 s, with no previous spikes in the preceding 45s; Faizo *et al.*, 2014). To examine the between-groups and within group

alterations in functional connectivity, activity in selected seed regions was covaried with activity in the rest of the brain during the resting state. Seed regions were selected using MNI coordinates selected from previous studies (Beucke *et al.*, 2014; Buckner *et al.*, 2009; Woodward *et al.*, 2011), which identified the critical nodes of each resting state network: DMN = posterior cingulate gyrus (PCC; -3 -48 30), SN = insula (INS; -38 16 -2), and DAN = intraparietal sulcus (IPS; -23 -70 46). For each seed, the average BOLD signal intensity was extracted and used for connectivity analyses, during rest, in both patients and controls, using the Partial Least Squares (PLS) analysis (McIntosh, Chau, & Protzner, 2004; McIntosh *et al.*, 1996; for a detailed tutorial and review of PLS, see Krishnan *et al.*, 2011).

PLS identifies significant whole-brain activity patterns related to task demands, measures of behavioural or anatomical covariates, or activity in a given seed region. Activity patterns are assessed across all brain voxels simultaneously, which is in contrast to mass-univariate analyses, which consider each voxel separately. PLS analysis uses singular value decomposition (SVD) of a single matrix, containing all participants' data to find a set of latent variables (LVs), which are mutually orthogonal dimensions that reduce the complexity of the data set. Each LV consists of a singular image of voxel saliences (*i.e.*, a spatiotemporal pattern of brain activity), a singular profile of task saliences (*i.e.*, a set of weights that indicate how brain activity in the singular image is related to the experimental conditions, functional seeds, or behavioural/anatomical covariates), and a singular value (*i.e.*, the amount of covariance accounted for by the LV). The significance of each LV is assessed by a permutation test, which determines the probability that a singular value from 500 random resamplings of the data is larger than the initially obtained value (McIntosh *et al.*, 1996). In addition to the permutation test, a second and independent step is to determine the reliability of the saliences (or weights) for each brain voxel that characterizes a given spatiotemporal pattern identified by the LVs. To do so, the standard error of each voxel's salience on each

LV is estimated by 100 bootstrap resampling steps (Efron & Tibshirani, 1985). Peak voxels with a bootstrap ratio (BSR; *i.e.*, salience/standard error) > 3.0 were considered to be reliable, as these approximate $p < 0.001$ (Sampson *et al.*, 1989). Because extraction of the LVs and corresponding brain images is done in a single analytic step, no correction for multiple comparisons is required.

Results

Between Groups Differences in Functional Connectivity

Default mode network

In healthy controls, functional connectivity analysis with PCC as the seed region showed significant connectivity between the typical DMN regions (Damoiseaux *et al.*, 2006; Koshino *et al.*, 2014): PCC, medial prefrontal cortex (PFC), middle temporal gyrus (MTG), parahippocampus, thalamus, precuneus, and bilateral inferior parietal lobule (IPL). In contrast to healthy controls, mTLE patients demonstrated a significantly different, left-lateralized connectivity pattern, showing dysconnectivity to medial PFC and parahippocampus, decreased connectivity to bilateral IPL and MTG, and increased connectivity to bilateral thalamus and precuneus ($p < 0.05$; **Fig 1A**).

Salience network

In healthy controls, functional connectivity analysis with INS as the seed region showed significant connectivity between the typical SN regions (Seeley *et al.*, 2007; Sridharan *et al.*, 2008): bilateral insula, ventral and dorsal PFC, dorsal anterior cingulate cortex (ACC), and cerebellum. In contrast, mTLE patients demonstrated significantly decreased connectivity to both insulae, dysconnectivity to dorsal ACC, as well as increased connectivity to thalamus ($p < 0.05$; **Fig 1B**).

Dorsal attentional network

In healthy controls, functional connectivity analysis with IPS as the seed region showed significant connectivity between the typical DAN regions (Fox *et al.*, 2005; Mantini *et al.*, 2007; Vossel *et al.*, 2014): bilateral frontoparietal regions: IPS, FEF, and dorsolateral PFC. In contrast, mTLE patients demonstrated significantly decreased connectivity to PFC regions, but increased connectivity to parietal areas ($p < 0.05$; **Fig 1C**).

[INSERT FIGURE 1 HERE]

Within mTLE Group Differences in Spike-Related Functional ConnectivityDefault mode network

During the pre-spike period, no significant connectivity pattern with PCC was found, compared to the rest epoch, suggesting that dysconnectivity to the DMN may play a role in the generation of interictal spikes. During the spike period, mTLE patients engaged the DMN, showing significant functional connectivity between PCC and prefrontal regions, compared to the rest epoch, which was characterized by dysconnectivity to PFC (**Fig 2A**).

Saliency network

During the pre-spike period, mTLE patients engaged the SN but showed decreased connectivity between the SN nodes, compared to the rest epoch (**Fig 2B**), suggesting that weaker connectivity within the SN may play a role in mediating interictal spikes. During the spike period, no significant connectivity pattern with INS was found, suggesting that the SN is not engaged during the spike period.

Dorsal attentional network

mTLE patients engaged the DAN during both pre-spike and spike periods, but with significant connectivity differences. During the pre-spike period, mTLE patients showed significantly decreased parietal connectivity, compared to the rest epoch, whereas during the spike period, the patients showed similar DAN connectivity to the rest epoch (**Fig 2C**).

[INSERT FIGURE 2 HERE]

Discussion

The objective of this study was to investigate mTLE-associated alterations in functional connectivity involving three resting state networks: DMN, SN and DAN, and their relationship to both interictal spikes and the pre-spike time epoch. Using simultaneous EEG-fMRI, we expanded on the findings of our previous study (Faizo *et al.*, 2014) in which we demonstrated a loss of bilateral hippocampal connectivity immediately prior to the appearance of interictal spikes. The results of the current study show significant mTLE-related alterations in functional connectivity of all three resting state networks compared to healthy controls. In contrast to the healthy cohort, the resting state networks of mTLE patients are characterized by dysconnectivity or decreased connectivity to prefrontal areas and increased connectivity to subcortical and posterior areas. In addition, there are significant differences in functional connectivity of the resting state networks between pre-spike and spike brain states in mTLE. In relation to the pre-spike period, mTLE patients engage the DAN and SN, but not DMN, and show, in contrast to the rest period, decreases in functional coherence within these networks. In relation to the spike period, mTLE patients engage the DMN and DAN, but not SN, and show, in contrast to the rest period, connectivity to prefrontal areas.

General alterations in resting state networks of mTLE patients

Compared to the healthy participants, all three resting state networks of mTLE patients show significant alterations during rest, both in terms of decreases and increases of functional connectivity between the networks' nodes. Specifically, disturbances in intrinsic activity of mTLE patients include dysconnectivity or decreased connectivity to prefrontal areas and increased connectivity to thalamus and posterior parietal areas. The prefrontal cortex has been implicated in complex and sequential brain functions, such as planning,

decision making, or response inhibition (Wood and Grafman, 2003). Our findings align with Yuan *et al.* (2013) who have recently reported decreased functional connectivity within the DMN of mTLE patients, and, especially, a lack of connectivity to the PFC. Whether these connectivity alterations reflect the impact of the underlying pathophysiology of mTLE or secondary seizure propagation is still unclear (Devinsky, 2005; Stretton & Thompson, 2012). Available evidence suggests that both mechanisms might be at play, as propagated activity from seizures and interictal spikes over a long period of time may cause significant functional changes in the DMN due to the PFC's connection to the compromised hippocampal complex by a direct neuroanatomical pathway (Thierry *et al.*, 2000). Other researchers have reported decreases in functional connectivity specifically between the hippocampus and PFC in mTLE patients (Pittau *et al.* 2012), as well as prefrontal hypometabolism and hypofunction in patients with intractable mTLE associated with cognitive decline (Takaya *et al.*, 2006).

In the SN, which subserves interoceptive awareness, environmental monitoring, and salient response selection (Taylor *et al.*, 2009), we found decreased functional connectivity between insular cortex and dorsal ACC, in line with recent work by Luo *et al.* (2014) who demonstrated that, in patients with childhood absence epilepsy who suffer from a loss of awareness, inability in identifying and manipulating salient stimuli is associated with abnormal connectivity between the main SN nodes. The structure and function of the SN might be directly impacted by mTLE-related hippocampal changes via the direct hippocampo-insular pathway, perhaps mediating a decline in associative learning, which is known to be directly proportional to the extent of hippocampal and parahippocampal damage in mTLE (Weniger *et al.*, 2004). As such, functional alterations within the SN may underlie specific mTLE deficits in the processing of salient information, such as newness (*e.g.*, detecting a new object in a well-known environment) or associative learning (*e.g.*, pairing between two stimuli).

In the DAN, which subserves goal-directed, top-down attentional processing (Corbetta & Shulman, 2002), we found decreased functional connectivity to PFC; specifically, to DAN's major prefrontal nodes, bilateral FEF, which play crucial role in controlling visual attention and eye movements (Schall, 2004). Our findings support existing behavioural evidence that demonstrates a decline in goal-driven attention during executive and working memory tasks in mTLE patients (Liao *et al.*, 2010, Zhang *et al.*, 2009). We suggest that the functional alterations in connectivity to the FEF might be a consequence of long-term injurious effects of seizures. More extensive evidence is needed to support this supposition conclusively; however, a recent study presents functional coherence of ictal spikes followed by fast rhythmic spikes in the frontal lobes of mTLE patient with epileptic nystagmus (Kim *et al.*, 2013).

In contrast to significant decreases in resting state connectivity, we observed significantly increased connectivity to the thalamus (DMN and SN) and precuneus (DMN and DAN). The thalamus is anatomically connected to the mesolimbic structures, namely the hippocampus, amygdala, and entorhinal cortex (Carlesimo *et al.*, 2011; Swenson, 2006) and the associated thalamo-cortical activity has been implicated in the regulation of consciousness (Min, 2010; Zhou *et al.*, 2011). Both the DMN and SN play a critical role in maintaining consciousness and awareness; as such, disturbances in functional connectivity within these networks might be associated with reductions or loss of awareness during seizures (Craig, 2009; Luo *et al.*, 2014). We suggest that the increased connectivity to the thalamus may serve as a compensatory mechanism that counters the functional alterations within the DMN and SN in mTLE patients. The precuneus, on the other hand, is known to be involved during orienting tasks (Kelley *et al.*, 2008, Yantis *et al.*, 2002) and is anatomically connected to the intraparietal sulcus, an area critically involved in visual and spatial processing (Cavanna and Trimble, 2006). Other cortical and sub-cortical projections to and

from the precuneus have been demonstrated, for instance to the thalami (Schmahmann and Pandya, 1990), claustrum, and brainstem (Yeterian and Pandya, 1993). This wide range of connections indicates the importance and complexity of the precuneus in regulating higher cognitive function, although its exact role and mechanism are not fully understood. Its abnormal connectivity to intraparietal sulcus in mTLE patients might, however, suggest compensatory recruitment in response to the lack of functional coherence within the DMN and DAN networks.

Specific mTLE alterations prior to and during interictal spikes

In the DMN of mTLE patients, two main findings were demonstrated in relation to the pre-spike and spike periods. Prior to the interictal spikes, we found evidence of dysconnectivity to the DMN, followed by the re-engagement of the DMN during the spike period, with increases of functional connectivity to the prefrontal cortex. Whilst earlier studies have linked the deactivation of the DMN to the interictal spikes (Fahoum *et al.*, 2012; Laufs *et al.*, 2007), our results specifically isolate these reductions in functional coherence within the DMN to the prespike period. This temporal epoch is likely to be critical to the genesis of spikes. Both SN and DAN were recruited prior to the interictal spikes, but the networks were characterized by significantly reduced functional coherence, compared to the rest period. These findings suggest that decreased functional connectivity in these two networks may play an important role in the generation of interictal spikes. Whilst the SN was deactivated during the spikes, in line with a recent study showing functional disruptions in the SN in relation to interictal spikes in children with intractable focal epilepsy (Ibrahim *et al.*, 2014), the DAN showed increases in functional connectivity comparable to that of the rest period. Together, these findings shed light on the role of oscillatory coherence in large-scale resting state networks in the modulation of interictal epileptiform spikes.

In conclusion, using simultaneous EEG-fMRI, we investigated alterations in functional connectivity within three resting state networks (DMN, SN, and DAN) of mTLE patients and the relation of the changing connectivity patterns to interictal epileptiform spikes. The findings show that, at rest, all three networks of mTLE patients are characterized by significant alterations in functional connectivity; specifically, dysconnectivity to prefrontal areas and increased connectivity to subcortical and posterior areas. Importantly, prior to the onset of interictal spikes, mTLE patients showed dysconnectivity to the DMN and decreased connectivity within the SN and DAN, demonstrating alterations in functional coherence that may be responsible for the generation of epileptiform activity. These functional changes may be related to epilepsy-related cognitive abnormalities, as higher cognitive functions rely heavily on the coordinated activity of all three resting state networks.

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Figure Captions

FIG 1. Between Groups Comparison: Functional Connectivity During Rest in A) the Default Mode Network; B) the Salience Network; C) the Dorsal Attentional Network.

FIG 2. Within TLE Patient Group Comparison: Functional Connectivity During Rest, Pre-Spike, and Spike Epochs in A) the Default Mode Network; B) the Salience Network; C) the Dorsal Attentional Network.

Table 1

PATIENTS	LATERALIZATION	AGE OF ONSET	DURATION (years)	CLINICAL MRI	TOTAL # SPIKES (across 6 runs)
1	R	21	12	HS	47
2	L	20	25	N	none
3	R	14	7	N	none
4	R	21	26	HS	52
5	R	21	2	N	none
6	L	30	25	N	50
7	L	17	6	N	24
8	R	16	14	HS	none
9	L	20	24	N	49
10	L	17	3	N	35
11	L	25	7	N	32
12	L	23	21	N	54
13	R	35	2	N	37
14	L	4	55	N	32
15	L	25	8	N	34

Table 1. Patient clinical details & spike distribution. **R** = right; **L** = left; **HS** = hippocampal sclerosis; **N** = normal; none = no spikes have been identified during EEG-fMRI.