Characterization of Seizure Generating and Propagating Regions in Human Focal Epilepsy with Resting State Functional Connectivity MRI

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Focal seizures represent aberrant electrical rhythms that originate in a specific cortical region, the seizure onset zone (SOZ), and propagate in a network of anatomically connected regions. MRI has emerged as a leading diagnostic methodology in the field of epilepsy over the last 20 years and has significantly impacted the surgical evaluation of patients with medically refractory focal epilepsy. However, thus far clinical MRI has largely been used as a structural tool, and there is an interest in developing MRI-based measures of seizure propagation and inter-regional connectivity. Resting state functional connectivity MRI (rsfMRI) allows identification of macroscopic brain networks based on functional connectivity, or co-activation, within regions (nodes) comprising the network. Functional connectivity (FC) describes the temporal correlation between spatially remote neurophysiologic events, and has been measured using different modalities including rsfMRI. Given the network characteristics of epilepsy, there has been an interest in applying rsfMRI to examine the seizure-generating regions of the brain and their functional interactions with other parts of the brain. In this review, we have focused on focal epilepsy, one of the two subtypes of epilepsy by physiology. Most studies of focal epilepsy, with notable exceptions, reported reduced functional connectivity of epilepsy producing and propagating regions, as well as associated reduction in functional connectivity of networks performing cognitive functions including memory, attention and language. We also reviewed reports of early applications of rsfMRI to lateralize/localize epileptic activity. Overall, resting state functional connectivity MRI (rsfMRI) has already contributed to a better understanding of epilepsy-related networks and promises to add to the non-invasive evaluation of patients with a non-lesional clinical MRI.

KEYWORDS: Resting Functional Connectivity MRI Focal Epilepsy.

INTRODUCTION

(i) Seizures and Epilepsy. An epileptic seizure is defined as a “transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain” [4]. Seizures could either consist of subjective symptoms or be associated with objective clinical manifestations such as behavioral arrest or convulsive motor activity, when most severe. Epilepsy is defined as a condition of recurrent unprovoked seizures and is the second most common neurological disease [5]. Epileptic syndromes are broadly defined by physiology as either focal (with seizure onset in one region of the brain) or generalized (with seizure onset involving synchronized involvement of bilateral brain networks). This review will be limited to focal epilepsy where seizure activity is traditionally believed to arise from a cortical region referred to as the seizure onset zone (SOZ) and subsequently propagate in a network of anatomically connected regions (seizure propagation networks). Given that scalp-recorded EEG is not always localizing (i.e., not always able to determine the lobe of the brain where seizure activity originates from), the most definitive methodology for locating the SOZ is EEG recording using intracranially placed electrodes (icEEG). However, icEEG is an invasive procedure that is only
performed for the purpose of locating the SOZ prior to surgery for medically refractory epilepsy. There is a need for non-invasive methods of identifying the SOZ. This goal has been significantly transformed with the advent of MRI.

(ii) Neuroimaging in Epilepsy Diagnosis. Structural MRI has emerged as a leading diagnostic tool in epilepsy over the last 20 years. However, there is an interest in developing functional imaging techniques that could detect seizure activity and/or characterize seizure propagation networks. Structural MRI identifies a lesion in only about 50% of cases of focal epilepsy, with the remaining 50% being referred to as non-lesional or cryptogenic. The identification of a structural and/or functional lesion is associated with a better prognosis for seizure freedom after epilepsy surgery [6,7]. There is therefore a need in our field to develop more sensitive, non-invasive procedures to help guide subsequent invasive investigations.
Task-activated blood oxygen level dependent (BOLD) functional MRI has successfully been used to identify areas of activation resulting from seizure activity (reviewed in Ref. [8]). A related MRI technique, resting states functional MRI (rsfMRI), uses the same MRI technique in the absence of a specific task [9, 10]. RsfMRI reveals functional linkages or co-activations between components of a brain network by examining the correlation of their respective BOLD signal over time. This property has been referred to as functional connectivity (FC) which describes the temporal correlation between spatially remote neurophysiologic events and has been measured using different modalities including rsfMRI [11, 12]. In the absence of a task, rsfMRI is surprisingly capable of revealing at rest many of the known brain networks including the motor network [9]. RsfMRI analysis is most often performed using a region of interest (ROI) approach where the average BOLD time course of all brain voxels within the ROI is statistically correlated with the time courses of all other brain voxels, generating a map of all brain regions which are statistically correlated, or functionally connected, with the ROI [10]. For example, seeding one node of the motor network, such as the motor cortex, has been shown to reveal the entire motor network using this analysis [9]. Given the power of this technique at identifying resting state networks (RSN), it is natural to address whether rsfMRI could detect seizure generating and propagating networks. In this manuscript, we will review the major findings of studies that measured FC of epilepsy-related networks. We will also consider the evidence behind the use of rsfMRI to lateralize/localize the seizure onset zone.

**REVIEW OF THE LITERATURE**

(i) Study of functional connectivity of epileptogenic regions using rsfMRI. This topic was addressed mainly in patients with temporal lobe epilepsy compared to controls. Functional connectivity (FC) was most often calculated as a correlation of the average BOLD time series between pairs of regions presumed to be involved in seizure generation (for example, Ref. [1]). FC was alternatively measured by generating whole-brain maps of all the voxels that are correlated with a specific region of interest (either derived from areas of seizure activation on EEG or by selecting a ROI in a region of presumed seizure onset such as the hippocampus) (for example, Refs. [13, 14]). Regardless of the approach, the most consistent finding is of reduced functional connectivity within epileptogenic regions [1, 13–16]. Interestingly, there was also evidence of increased functional connectivity between certain regions in the contralateral, non-epileptic hemisphere, hypothesized to represent adaptive compensatory mechanisms (Fig. 1) [1, 17]. The only report of increased BOLD FC in focal epilepsy was in the special situation of epilepsy resulting from nodular heterotopia, a migrational disorder resulting from aberrantly positioned subcortical aggregates of neurons [18]. There was increased FC between epileptic heterotopic nodules and their overlying cortex when compared to other nodule-cortex pairs that were not involved in seizure generation. Overall, the emerging theme of most studies was of reduced functional connectivity within seizure-generating regions, using the modality of rsfMRI, with the exception of the special case of nodular heterotopias.

The role of the contralateral side was explored by probing interhemispheric interactions between the epileptic and the non-epileptic hippocampus using rsfMRI [19]. Early in the course of the epilepsy, there is disruption of connectivity between the two sides; however, over time, the non-epileptic hippocampus starts influencing the epileptic side, a directional relationship determined using methods of effective connectivity (which add to functional connectivity by estimating directionality of influence of one region over another). This suggests that rsfMRI is able to monitor the plasticity of epileptogenesis over time, and that it could potentially serve as an imaging marker. Although our focus in this review is on the FC of the epileptic region itself, investigators also explored the interactions between the epileptic region and certain resting state networks. For example, the epileptogenic region in temporal lobe epilepsy was found to also be less connected to other networks including the default mode network (DMN), a network of regions that is active during resting wakefulness, as well as to dopaminergic and mesolimbic regions [14]. One study was unique in using intracranial EEG data, considered the gold standard for localization of the seizure onset zone, to identify regions of interest for probing rsfMRI data [3]. In addition to measuring FC using rsfMRI signals, the authors also measured FC using intracranial EEG (icEEG) data. Consistent with most other studies using rsfMRI to measure FC in patients with focal epilepsy, they found reduced connectivity within some of the regions generating epileptiform activity when using rsfMRI to measure FC. However, in parallel they also found the opposite result, i.e., increased connectivity, when measuring the FC of the same regions using icEEG data (Fig. 2). These apparently discordant findings suggested to the authors that FC, measured by these two distinct modalities, was not equivalent.

(ii) Using rsfMRI to lateralize or localize seizure activity. Lateralizing seizure onset, i.e., determining if it is of right or left hemisphere onset, and localizing the lobe of onset is an important clinical task, especially in the context of presurgical evaluation for medically refractory epilepsy. The more information that could be obtained from non-invasive procedures, the easier it is to find the seizure onset zone and to be able to offer the patient a potentially curative surgery. Given the above findings of altered FC in epileptogenic regions, several groups addressed the clinical utility of rsfMRI in lateralizing and localizing seizure onset. One report identified areas of activation generated by frequent interictal epileptiform
Characterization of Seizure Generating and Propagating Regions in Human Focal Epilepsy with Resting State Functional Connectivity MRI

Karkar et al.

REVIEW

Fig. 1. Decreased inter-regional functional connectivity between certain region pairs within the epileptic limbic network in patients with left temporal lobe epilepsy. Right. Note reduced FC between the entorhinal cortex and anterior hippocampus and between the anterior hippocampus and posterior hippocampus. Left. Note increased FC between the anterior hippocampus and posterior hippocampus, hypothesized to represent an adaptive compensatory mechanism in the non-epileptic hemisphere. Reprinted with permission from [1], G. Bettus, et al., Decreased basal fMRI functional connectivity in epileptogenic networks and contralateral compensatory mechanisms. Hum. Brain Mapp. 30, 1580 (2009). © 2009.

Fig. 2. Modality-dependent measures of functional connectivity. (A) ROIs assigned in each patient based on epileptic activity (each dot is an icEEG electrode location with the color codes corresponding to epileptic activity; red being the most active [seizure onset region] and blue the least active). The figure shows the within-zone pairing used to calculate functional connectivity. (B) Functional connectivity measured using icEEG (top) and rsfMRI data (bottom). Double lines indicate increased connectivity and dotted lines reduced connectivity. In the top illustration, note relatively increased icEEG FC within the epileptiform-active regions (red, yellow). In the bottom illustration, note reduced rsfMRI FC within the IZ2 (intermediately active region). Both modalities showed reduced inter-zone FC between IZ1–IZ2. The red arrow indicates a significant directional index (effective connectivity), found to be present with either modality, showing that the epileptogenic regions exert an influence over the non-epileptic areas, even when overt seizure activity is not occurring. Reprinted with permission from [3], G. Bettus, et al., Interictal functional connectivity of human epileptic networks assessed by intracerebral EEG and BOLD signal fluctuations. PLoS One 6, e20071 (2011). © 2011.
discharges, without the use of EEG, by analyzing resting rsfMRI [20]. The authors had been motivated by a prior general finding of anti-correlation between activity of the default mode network (DMN) and areas of task activation regardless of cause (including activations resulting from subclinical epileptiform activity). Specifically, they identified regions whose time-course was anti-correlated with that of the posterior cingulate cortex (PCC), one of the nodes of the DMN. These regions, whose activation was associated with transient deactivation of the PCC, coincided with regions of suspected seizure activity based on clinical testing. Although promising, only two subjects with epilepsy were studied and correlation with simultaneous EEG-fMRI was not performed to confirm the presumed concordance. Another group used a ROI-based approach to seed rsfMRI data in mesial TLE.

Fig. 3. rsfMRI, independent of EEG information, identifies regions of increased connectivity that overlap with epileptogenic regions identified separately with iEEG. Each row represents data from one patient. The first three columns illustrate the functional abnormality and consist of Z score maps superimposed on anatomical images in 3 dimensions. The Z scores represent functional abnormality scores for each voxel (derived by comparison of global connectivity at each voxel with the average of a normative sample). Local and remote connectivity measures were obtained (red versus blue respectively, each with its own scale). Superimposing the abnormality maps over the epileptiform-active electrodes, as illustrated in column 4, identifies at least some overlap in all 5 patients. The blue circles in the map indicate the iEEG electrodes of seizure onset, whereas the green circles indicate interictal epileptiform discharges. Reprinted with permission from [2], S. M. Stufflebeam, et al., Localization of focal epileptic discharges using functional connectivity magnetic resonance imaging. J. Neurosurg 114, 1693 (2011). © 2011.
and concluded that the presence of functional connectivity increases in the non-epileptic side was the most specific marker of epileptogenic zone localization (with a sensitivity of 64% and specificity of 91%). This high specificity, stemming from the finding that FC increases were limited to the non-epileptic side, justified the conclusion that a single rsfMRI study could be useful in presurgical assessment of mesial TLE patients [21]. However, the study also concluded that a direct examination of the epileptic hemisphere using rsfMRI is not able to determine the regions involved in seizure generation given the finding of bilateral decreases in FC. Another study explored the use of rsfMRI to predict seizure freedom after epilepsy surgery; after calculating whole-brain connectivity maps of the region of maximum epileptiform activity (as determined from a prior simultaneous EEG/fMRI study of interictal epileptiform activity) and assigning them laterality indices, they concluded that the more laterialized maps (i.e., those with fewer bilateral connections) predicted higher seizure freedom after temporal lobe surgery [22]. Specifically, patients in the seizure-recurrence group had less laterализed functional connectivity compared to patients in the seizure-free group ($t_{16} = 2.3, p < 0.05$). Given the goal of contributing to non-invasive presurgical evaluation and the high spatial resolution of simultaneous EEG/fMRI, the authors understandably used pre-surgical interictal EEG/fMRI data as regions of interest. However, interictal discharges are not always concordant with the seizure onset zone, and it would be interesting to generate connectivity maps using ictal EEG information in patients where the SOZ is well localized (from ictal scalp or invasive video/EEG data). Separately, a thalamic-hippocampal connectivity link, derived from a seed-based analysis of the right hippocampus in temporal lobe epilepsy (TLE) patients, was identified as an indicator of lateralization of TLE [23]. Comparing right hippocampus-derived connectivity maps among three groups (left TLE, right TLE, and controls), the authors reported connectivity between the right hippocampus and the ventral lateral nucleus of the right thalamus to be significantly different between the left TLE and right TLE groups by ANOVA ($p < 0.0001$). The authors therefore raised the potential of using this right hippocampal-thalamic link as a lateralizing measure (left versus right onset). However, as the authors noted, further validation in a larger cohort is required. Finally, a data-driven approach to localize the seizure onset zone used a global FC analysis [2]. For each patient, the authors generated a global FC map where each voxel was assigned a connectivity value that was the sum of its interactions (selecting for increased FC above a threshold of 0.25) with all other voxels at two proximity levels (remote versus local). For statistical analysis, the authors then calculated functional abnormality scores ($Z$ values) for each voxel (derived by comparison with a normative sample) as shown in Figure 3. The regions identified using rsfMRI analysis overlapped the epileptogenic regions as later independently identified with iEEG in all five patients (Fig. 3). Although the sample was small, the estimated diagnostic accuracy was a sensitivity of 0.83 and specificity of 0.91. The authors hypothesized these FC abnormalities to result from epileptic activity given the overlap with intracranial EEG, however acknowledged the possibility of underlying epilepsy-related structural changes as accounting for the concordance with the SOZ; this is a question that could be addressed with simultaneous EEG/fMRI. Taken together, there are reports suggesting successful use of rsfMRI to lateralize and/or localize epileptogenic regions in focal epilepsy, though the studies mainly consisted of small patient cohorts.

**DISCUSSION AND FUTURE DIRECTIONS**

(i) Reduced functional connectivity using rsfMRI in seizure-generating regions, with exceptions. Most studies, mainly of temporal lobe epilepsy, reported reduced BOLD functional connectivity in seizure-producing regions. However, there are some notable exceptions [2, 18] that need to be reconciled. In particular, one of the studies identified regions of increased FC that surprisingly overlapped with the seizure onset region, as independently identified with EEG [2]. However, as the authors suggested, the increased functional connectivity could be the result of subclinical epileptiform activity (could not be verified as simultaneous EEG/fMRI was not used). Increased FC was also reported between subcortical heterotopic nodes and the corresponding overlying cortex [18]. Some of these differences could be methodological; Stufflebeam et al. [2] used a data-driven connectivity model, whereas most of the other studies used a hypothesis-driven ROI-based approach. The case of nodular heterotopias, resulting from developmental migrational defects, may represent a special case with increased functional connectivity in epileptogenic region. The direction of FC (increased versus reduced) likely depends on the epileptic state of the network (ictal versus interictal); most studies did not control for subclinical interictal epileptiform activity with exceptions [14]. Future studies, using simultaneous EEG/fMRI, are needed in order to address this question.

(ii) EEG versus rsfMRI measures of FC. Functional connectivity has recently become most often associated with fMRI, even though there is a parallel literature exploring FC using other modalities including EEG. There are several EEG studies of FC that measured an increase in EEG-based FC in epileptogenic regions (for example, Refs. [24–26]). The apparent discrepancy between FC measured using EEG versus rsfMRI [3], should be noted in this regard. As an explanation, the authors suggested that the two modalities measure different phenomena that are occurring at different time scales: BOLD-based FC as a marker of network health during the interictal period, whereas iEEG as a measure...
of region-specific pathological hypersynchrony characteristic of seizure activity. In support of the concept that BOLD-based FC is correlated with network integrity in epilepsy is the consistent finding of a correlation between reduced functional connectivity of certain resting state networks (RSN) and cognitive dysfunction in domains such as memory and language [15, 17, 27–38]. Of special interest to epilepsy surgery in this regard is the use of

![Image of brain with ROIs and icEEG data]

**Fig. 4.** Probing rsfMRI data using ROIs placed along seizure propagation pathways, with directionality derived from icEEG. The following describes analysis of two patients with extra-temporal epilepsy. (A)–(B). Determination of the seizure onset zone. (A) Seizure-onset zone (SOZ) identified using subdurally placed electrodes (in this case in the superior parietal region, covered by a subset of the red electrodes); electrode locations are registered from CT to MNI space. (B) icEEG recordings indicate the region of seizure onset. Red arrow points to 4 electrode channels revealing repetitive sharp waves, representing the seizure rhythm. (C)–(E). ROI Placement and calculation of inter-regional functional connectivity. (C) Patient 1: ROI placed within the SOZ (green) and in the homologous region of the left hemisphere (red). (D) Other ROIs placed in locations of interest, determined from seizure propagation information derived from icEEG (A)–(B). (E) Inter-regional functional connectivity values from two patients, comparing the epileptic and non-epileptic hemispheres and showing relative differences (a method for normalization to allow group comparisons). Each functional connectivity pair in the epileptic hemisphere is compared with the homologous pair from the non-epileptic hemisphere (SOZ, seizure onset zone; N1, first node of seizure propagation; N2, secondary node of seizure propagation; UI, uninvolved region). Figure from authors’ preliminary data.
rsfMRI to examine post-operative functional reorganization after surgery, with findings of correlation between post-operative adaptive plasticity and the establishment and/or reinforcement of certain functional connections [36, 38].

(iii) Future directions. There are future questions to explore. One of the still open questions is whether rsfMRI could reveal epileptic networks that may be distinct from normal, physiological networks. So far, data is pointing to altered functional connectivity within existing networks. Furthermore, what is referred to as epileptic networks may not necessarily represent networks in the typical sense (i.e., resting state networks (RSN) such as the motor network or DMN), but may rather refer to pathways of propagation of seizure activity; a clarification of the terminology is therefore needed. Additional investigations, using simultaneous EEG/fMRI, should help address the question of how seizure activity modulates functional connectivity of epilepsy producing networks; although a difficult task to achieve in the MRI scanner, it is possible to accomplish in cases of non-convulsive focal seizures, as has been accomplished in patients with non-convulsive generalized epilepsy [39].

Furthermore, exploring functional connectivity of actual seizure-propagation pathways, as determined by intracranial EEG, is another potentially promising direction; we are currently conducting a study designed to address this question (Fig. 4). Most of the studies done to date have used either empiric regions of interest or those derived from interictal scalp EEG. In our study, we are using ROIs derived from seizure propagation data. We have asked whether seizure propagation networks could be revealed using MRI in focal, extra-temporal epilepsy. We have probed rsfMRI data using regions of interest along seizure propagation pathways, with directionality derived from EEG. As subjects, we are studying patients with medically refractory focal epilepsy of suspected extra-temporal origin undergoing a pre-surgical evaluation with iEEG. To date, we studied eight patients, all of whom underwent rsfMRI before invasive video/EEG monitoring. Figure 4 has results of analysis from two patients. Although a group analysis has not been performed to date, there is preliminary evidence suggesting reduced interictal functional connectivity within the seizure propagation network.

The majority of the studies to-date was either performed in generalized epilepsy syndromes (not the focus of this review) or in temporal lobe epilepsy (a subtype of focal epilepsy). Additional future studies are also needed of patients with less common types of focal epilepsy, especially patients with a normal clinical MRI. Such patients with non-lesional clinical MRIs are ones where non-invasive diagnosis is most needed and could have the most impact, especially on the lives of patients with medically refractory epilepsy being considered for epilepsy surgery.

CONCLUSIONS

Most studies of focal epilepsy, with notable exceptions, reported reduced functional connectivity of seizure-generating regions, as well as associated reduction in functional connectivity of networks performing cognitive functions. Furthermore, there is early evidence supporting the exploration of rsfMRI as a non-invasive diagnostic tool to lateralize and localize seizure activity. Of interest are also reports of the use of rsfMRI to longitudinally follow the course of epilepsy over time and to measure post-operative plasticity after epilepsy surgery. Overall, resting state functional connectivity MRI (rsfMRI) is contributing to a better understanding of epilepsy-related networks and promises to add to the non-invasive evaluation of patients with focal epilepsy and a non-lesional clinical MRI.

Conflict of Interest

There is no conflict of interest involving any of the authors.

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