Published in final edited form as:

Curr Opin Neurol. 2013 April ; 26(2): 186–194. doi:10.1097/WCO.0b013e32835ee5b8.

Connectomics and epilepsy

Jerome Engel Jr.^{a,b,c,e}, Paul M. Thompson^{a,c,e}, John M. Stern^a, Richard J. Staba^a, Anatol Bragin^{a,e}, and Istvan Mody^{a,d,e}

^aDepartment of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

^bDepartment of Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

^cDepartment of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

^dDepartment of Physiology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

^eThe Brain Research Institute, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

Abstract

Purpose of review—Tremendous advances have occurred in recent years in elucidating basic mechanisms of epilepsy at the level of ion channels and neurotransmitters. Epilepsy, however, is ultimately a disease of functionally and/or structurally aberrant connections between neurons and groups of neurons at the systems level. Recent advances in neuroimaging and electrophysiology now make it possible to investigate structural and functional connectivity of the entire brain, and these techniques are currently being used to investigate diseases that manifest as global disturbances of brain function. Epilepsy is such a disease, and our understanding of the mechanisms underlying the development of epilepsy and the generation of epileptic seizures will undoubtedly benefit from research utilizing these connectomic approaches.

Recent findings—MRI using diffusion tensor imaging provides structural information, whereas functional MRI and electroencephalography provide functional information about connectivity at the whole brain level. Optogenetics, tracers, electrophysiological approaches, and calcium imaging provide connectivity information at the level of local circuits. These approaches are revealing important neuronal network disturbances underlying epileptic abnormalities.

Summary—An understanding of the fundamental mechanisms underlying the development of epilepsy and the generation of epileptic seizures will require delineation of the aberrant functional

and structural connections of the whole brain. The field of connectomics now provides approaches to accomplish this.

Keywords

epilepsy; functional connectivity; local circuits; structural connectivity; whole brain

INTRODUCTION

Considerable research over the past several decades has focused on elucidating basic mechanisms of epileptogenesis (the development and progression of an epilepsy condition), and epileptogenicity (the capacity to generate spontaneous seizures, also referred to as ictogenesis). There have been tremendous advances in understanding the genetic and molecular substrates of epileptically abnormal neuronal excitability at the level of neurotransmitter functions, ion channels, and intracellular processes. Further research with transgenic animal preparations, viral transfection, the pharmacological manipulation of genetic expression, and the use of induced pluripotent stem cells promises even more dramatic insights in the near future. This research direction is yielding novel targets for antiepileptogenic and antiseizure drugs and other interventions, but to date has provided little insight into how networks of neurons influenced by these molecular disturbances are transformed to produce a chronic state of epilepsy, or to generate spontaneous seizures.

Epileptic seizures reflect abnormal neuronal synchronization as much as alterations in excitability, and synchronization can only be studied at the level of neuronal networks. Such systems-level investigations can range from local circuits within an epileptogenic structure such as the hippocampus, to neuronal connections involving the entire brain. There is increasing evidence that even focal epilepsy disorders are associated with highly diffuse, often bilateral, structural and functional disturbances, which contribute importantly to the development and maintenance of chronic epilepsy.

Tremendous advances in a new field, referred to as connectomics, are bringing a wide variety of technologies to bear on understanding the interactions among neurons and among groups of neurons widely spaced throughout the brain, in the expression of emergent normal behaviors [1[¶]] (www.humanconnectome.org). These approaches are now also being applied to certain disorders that manifest as a result of global disturbances of the brain. Epilepsy is a prototype of such diseases and epilepsy research should benefit greatly from application of new developments in connectomics.

This review will consider new technologies of connectomics at the whole brain and local circuit levels. Because these technologies have not yet been applied, in most cases, to studies of epilepsy, this review will also present some speculation on types of investigations that should be carried out [2[¶]]. The whole brain level will include structural connections as demonstrated by diffusion tensor imaging (DTI) and other MRI approaches to demonstrate white matter tracts, as well as functional studies including the various approaches to resting functional MRI (fMRI), invasive, and noninvasive electroencephalography (EEG). At the local circuit level, optogenetics is an improvement on electrical stimulation tractography, as

it permits studies of the effects of stimulation of specific types of neurons. Various tracers, other electrophysiological techniques, and calcium imaging will also be discussed.

WHOLE BRAIN LEVEL

Both structural and functional connectivity can now be studied at the level of the whole brain.

Structural connectivity

Diffusion imaging is a variant of standard MRI that is sensitive to water diffusion in the living brain [3,4]. Fiber tracts constrain water diffusion to occur primarily along the direction of the tract, and cell membranes and myelin sheaths inhibit diffusion orthogonal to the axon. By following the dominant directions of diffusion across the brain, whole-brain fiber tracking methods can reconstruct three-dimensional fiber pathways throughout the brain (Fig. 1). More sophisticated scanning methods – such as high-angular resolution diffusion imaging – can be used to disentangle fibers that cross or mix, leading to a complex, reconstructed three-dimensional network of intersecting neural pathways and circuits. In one type of connectivity analysis, a standard anatomical MRI scan is also collected, and computational analysis software is used to automatically subdivide the entire cortical mantle into gyral subdivisions. Fiber tracking software can then be used to track, count, and quantitatively characterize all the detected fibers that run between all the parcellated cortical regions. In a typical anatomical connectivity analysis with diffusion MRI, a ‘connectivity matrix’ is compiled to represent the density or integrity of fibers passing between all pairs of cortical and subcortical regions (Fig. 1). This two-dimensional image, or ‘connectome’, is essentially a ‘fingerprint’ of an individual’s neural connectivity, as seen with diffusion MRI. Because the connectome is essentially a two-dimensional image, image-based statistics can be applied to identify tracts or networks that are statistically different in cohorts of patients relative to controls. Some caveats are needed: diffusion MRI only picks up the largest fiber bundles, and inevitably misses functionally important pathways, given the standard 2-mm resolution of MRI. The anatomical resolution of fiber mapping is limited by the magnetic field strength of the scanner, as well as the angular resolution of the scanning protocol, so some studies have used higher field imaging – at magnetic fields of 7-Tesla or higher – to assess the microcircuitry of the hippocampus, as well as hippocampal neurite loss *in vivo* [5]. In clinical studies using these techniques, Vollmar *et al.* [6] studied a cohort of 29 patients with juvenile myoclonic epilepsy (JME) using DTI and functional neuroimaging, and found alterations in microstructural connectivity of the mesial frontal region that may contribute to the ‘cognitive triggering’ of motor seizures in JME. In a related study, Vuilleumoz *et al.* [7] argued that impaired white matter connectivity may contribute to functional frontal lobe abnormalities in JME. In focal frontal lobe epilepsy, the structural connectivity of the supplementary motor area was preserved, suggesting a robust motor network that is not compromised by longstanding epilepsy involving the medial frontal lobes. Holt *et al.* [8] mapped the superior longitudinal fasciculus (SLF) in children with drug-resistant partial epilepsy, and found lower fiber integrity in the SLF, but not in the cingulum, perhaps contributing to functional abnormalities in the patients. Pushing the resolution of DTI down to 50 microns using a 14-

Tesla scanner, Shepherd *et al.* [9] demonstrated many components of the trisynaptic intrahippocampal pathway, including perforant and Schaffer fibers, revealing connectivity *in vivo* in the rat hippocampus. Li *et al.* [10] targeted the cerebellum – a tricky area to resolve with DTI – and found cerebellar fiber integrity was impaired in patients with idiopathic generalized epilepsy.

Diffusion imaging may also be used in experimental animals, to survey the extent of white matter impairment. In one study, Otte *et al.* [11] applied diffusion tensor imaging in a rat model of focal epilepsy; they found widespread reductions in white matter integrity in diffuse brain regions well beyond the vicinity of the epileptic focus, which perhaps impaired the efficiency of functional networks.

Graph theory has also been widely used to summarize key properties of network organization – such as network efficiency, and clustering [12]. These measures can readily be computed from an individual's connectivity matrix, and ongoing work is defining the normal range, and normal trajectory of these network metrics in large cohorts of patients across the lifespan, to help define criteria for clinically relevant deviations [13,14]. In one study, Bonilha *et al.* [15] found evidence to suggest that seizure induced neuronal loss and axonal damage in medial temporal lobe epilepsy (MTLE) may lead to the development of aberrant connections between limbic structures. This may eventually result in the reorganization of the limbic network. In 12 patients with unilateral MTLE and hippocampal sclerosis, they found that patients showed a regional reduction in temporal lobe fiber density compared with controls, as well as abnormal limbic network clustering, and abnormal nodal efficiency, degree and clustering coefficient in the ipsilateral insula, superior temporal region and thalamus. These and other studies suggest that measures of network architecture and organization – as well as more traditional measures of individual tract integrity – will help in understanding aberrant brain connectivity, how it contributes to clinical presentation and prognosis, and factors that affect network organization in chronic disease.

Functional connectivity

Functional connectivity at the whole brain level can be studied with both fMRI and EEG.

Functional MRI—Cerebral connectivity can be investigated with fMRI by determining the extent of correspondence of activity across anatomic regions. Essentially, regions that have similarly fluctuating signals can be considered to be functionally related, either by one region influencing another or both being influenced by a third region. Overall, fMRI measures of connectivity are inferior to structural MRI in spatial resolution and considerably inferior to EEG in temporal resolution. Nevertheless, fMRI connectivity is valuable by providing a tomographic, metabolism-based result.

Determining connectivity with fMRI can be based on either a seed region of interest or a spatial independent component analysis (ICA) [16] (Fig. 2). Seed-based connectivity analysis begins with defining a region or collection of regions, and the analysis then establishes which other clusters of voxels meet the statistical threshold of similar fluctuation. The selection of seed location is based upon preexisting understanding of localized function and produces an investigation of a specific hypothesis, so it can be biased toward established

notions of a region's functional importance. The ICA approach does not depend on a selected region. Instead, it identifies regions (components) that collectively are distinct from the remainder of the brain within statistical thresholds for independence, and connectivity is apparent between the noncontiguous regions within a component. The ICA approach is free of established notions, so it is less likely to be influenced by investigator bias. Overall, each technique has advantages.

Functional connectivity abnormality due to unilateral MTLE has been investigated using both techniques. fMRI seed-based analyses have included seeds within the amygdala and hippocampus ipsilateral to the epileptogenic side. This has identified decreased connectivity to the contralateral anterior temporal lobe, ventromesial prefrontal region (dopaminergic mesolimbic network), and portions of the default mode network (DMN) [17,18^{••}]. Specifically, DMN connectivity is reduced to the posterior cingulate, precuneus, mesial prefrontal, and superior frontal cortex. Seeds within the anterior and posterior medial DMN identified decreased connectivity between the anterior and posterior DMN in unilateral MTLE and a difference in the DMN extent with an abnormally broad distribution of the frontal DMN in left MTLE [19^{*}]. Unilateral connectivity also differs in MTLE, as identified in the connectivity of five seeds within an epileptogenic region and five additional seeds that are contralaterally homotopic [20]. Even on the level of individual patients, the epileptic side had decreased connectivity across the unilateral seeds and the nonepileptic side had increased connectivity, compared with controls. Similar decreased connectivity was found using the ICA approach and the abnormality was associated with structural changes in the local gray and white matter [21]. Overall, these studies have identified enduring dysfunction associated with MTLE that is independent of the time of seizure occurrence and extends beyond the recognized epileptogenic region. This may lead to a better understanding of the cognitive and behavioral problems commonly associated with MTLE, and may potentially lead to a better understanding of the anatomy of the epileptic network, and improved surgical interventions.

Electroencephalography—Although MRI studies have identified gray and white matter structural changes that could reflect the anatomical substrates that support epilepsy, it remains to be determined how and to what extent structural disturbances give rise to abnormal network activity or which types of damage are epileptogenic and which ones are not. Modalities that measure functional connectivity can be combined with structural studies to investigate the association between network structure and function. The whole-brain spatial detail of fMRI combined with the temporal resolution of EEG, which can capture the rapid cellular processes associated with epileptiform discharges, can be used to investigate the spatial extent of the epileptogenic network. Recent work has focused on developing well tolerated fMRI protocols in patients with intracranial electrodes that could provide depth EEG data and neurovascular function [22,23,24^{*},25].

Functional connectivity studies using scalp EEG or intracranial grid and depth electrode-recorded EEG typically quantify the statistical interdependence between EEG signals captured from electrodes overlying or within different brain areas. Linear analysis between two EEG signals in the time-domain and frequency-domain include cross-correlation and coherence, respectively, whereas nonlinear components of EEG signals can be evaluated

using methods such as mutual information, phase synchronization and some forms of regression analysis. Such methods have been used to quantify the epileptogenicity of temporal lobe networks capable of generating spontaneous seizures [26,27], whereas information flow analysis of intracranial ictal EEG signals has been used to successfully predict electrode(s) where seizures begin [28–30]. Similar analyses have been applied to interictal EEG data to evaluate functional connectivity within and outside of the seizure onset zone in patients with different types of epilepsy [31–33]. A consistent finding among these studies is that the extent of functional connectivity is frequency-dependent and influenced by brain areas, location of the seizure onset zone, and type of epilepsy.

Multisite electrode recordings and measures of EEG signal interdependence can also be represented as basic elements of a graph. For example, electrode contacts positioned in anatomic brain areas typically denote vertices or ‘nodes’ and the extent of EEG connectivity (i.e., magnitude of cross-correlation, coherence, etc) reflects links between nodes called ‘edges’. Graphs based on EEG recordings can then be analyzed using standard graph metrics (e.g., edge density, path length, clustering coefficient, etc) that quantify network structure and could provide spatial information on brain areas capable of generating spontaneous seizures, that is, epileptogenic networks. The construction of graphs can be affected by many variables, including, for example, the number, location, and density of electrodes, choice of reference, and methods and rules quantifying connectedness. A recent EEG study in patients found evidence for increased regularity of networks in the epileptic brain compared with normal brain [34]. This was consistent with structural MRI data that indicated greater regularity or lattice-like network connectivity in patients with epilepsy, particularly in limbic regions associated with temporal lobe epilepsy, compared with controls [35]. How such a network contributes to seizure genesis is not clear, but a computer modeling study suggests structural alterations are not required to generate seizure-like activity [36]. Interestingly, computer simulations with reduced structural network connections could give rise to focal or generalized seizure-like activity that suggests some types of connections promote ictal activity, although others might suppress or limit the spread of activity to remote brain areas.

LOCAL CIRCUIT LEVEL

The function of the central nervous system cannot be understood without knowing the activities of large interconnected neuronal ensembles at a high temporal resolution. Membrane potential (V_m) changes and oscillations in individual neurons and groups of specific interconnected cells give rise to the complex activities of neuronal networks, which ultimately underlie behavior. Therefore, one of the major challenges in neuroscience is to record simultaneously the activation of various connected brain regions and circuits. Presently we possess reasonable anatomical information about the interconnections between various parts of the brains or nervous systems of many model organisms. However, just as knowing the map of the railway tracks of a region does not necessarily provide insight into the origin and destination of the trains, their speed or their timetable, a brain ‘connectome’ without functional details provides little information on the detailed workings of the complex system. Moreover, the brain is a plastic organ, and therefore the trains carrying information may take preferential tracks or even build new tracks that may not have been

mapped anatomically. To understand the complexities of the functioning brain, it is a prerequisite to record electrical activity in the interconnected networks of its active components.

Microelectrodes and slow large-scale activity detectors

Electrodes have been the workhorses of physiology, but as a general rule they cannot monitor large populations of individually identified cells. Microelectrode recordings of local field potentials generated by the surrounding neurons reflect, at best, the discharges of cells in a sphere of a radius of about 140 μm (a volume of 0.0065 μl) [37]. To measure neuronal activity in a mere 1.3% of a single mouse's total brain volume of approximately 500 μl , 1000 electrodes would have to be inserted in as many different locations, causing harsh tissue damage. Although spike sorting may be able to distinguish between action potential waveforms of thousands of cells [38,39], there are a myriad of other logistical problems in large scale recordings using electrical contacts. One of the most important drawbacks is the lack of proper identification of the recorded cells. Nonelectrical methods also exist to assess large-scale neuronal activity. Blood oxygenation level dependent fMRI, activity-related gene or protein expression levels have all been used, but these markers have various degrees of signal loss, are heterogeneous among different neurons, and most importantly, by lagging seconds, minutes, or even hours behind the cellular activity, they report at timescale orders of magnitude slower than the natural activity of brain cells. Lately, opto-fMRI has been used to assess the propagation of triggered neuronal activity in the brain [40,41] (Fig. 3); however, there are a number of disadvantages of this technique, starting with its poor temporal resolution (>10 s) when it comes to whole brain imaging.

Cellular Ca^{2+} measurements

Measuring changes in Ca^{2+} by optical means is by far the most widely used method in biology to assess cellular activity, and thus indirectly measure changes in V_m [43,44]. Ca^{2+} indicators are bright, come in a wide variety of colors and have great signal to noise ratios ($R_{S/N}$). The dyes can be easily loaded into cells in a noninvasive manner using their AM-ester forms. In addition to dyes, many fluorescent Ca^{2+} -indicator proteins have been developed and are being improved upon by several groups [45–48]. Their obvious advantage is that they can be genetically encoded [hence the name genetically encoded Ca^{2+} -indicators (GECI)] and consequently can be expressed in genetically defined subpopulations of cells. Indeed, Ca^{2+} reporting devices have been successfully used to measure activity in a large number of cells within a network. Ca^{2+} dyes with a low affinity to Ca^{2+} and a fast on-rate for Ca^{2+} binding can provide a relatively accurate, albeit indirect, measure of action potential firing in multiple individual cells of a network [48–54]. In contrast to Ca^{2+} dyes, presently there are no available GECI that bind Ca^{2+} with a low affinity. Therefore, the highly desirable and advantageous genetic encoding properties of the GECI are offset by their inability to measure the fast Ca^{2+} transients associated with single action potentials. The use of high Ca^{2+} affinity GECI has several additional major drawbacks. First, Ca^{2+} signals are inherently slower than action potentials, making it impossible to distinguish between individual action potentials occurring at high frequencies. Hence, it is impossible to capture the finer details of information encoded by high-frequency spiking; at lower firing frequencies, the timing of action potentials between different cells can only be measured at a

low temporal resolution (several ms). Second, GECI with high affinity for Ca^{2+} can easily be saturated, thus preventing the resolution of individual events once the signal reaches the saturation point. Third, by definition, Ca^{2+} -measuring tools will only report on cellular activities that trigger changes in Ca^{2+} concentrations. Consequently, hyperpolarizations and subthreshold depolarizations will remain unregistered, thus precluding the detection of countless events below the threshold of action potential firing or Ca^{2+} entry. Finally, Ca^{2+} dynamics are shaped by the complex interactions between Ca^{2+} channels, pumps, and buffers. As GECI are high affinity Ca^{2+} buffers, their presence will significantly alter the dynamics of intracellular Ca^{2+} signaling and with that the entire cellular signal transduction process.

Optical measurements of V_m

Unfortunately, direct V_m imaging has considerably lagged behind Ca^{2+} imaging because measuring V_m is associated with substantial biophysical hurdles [17]. One of the most important hurdles is the small space over which V_m can be measured in the cell. Although the electric field over the thin (~4 nm) membrane is huge (10^7 – 10^8 V/m), outside of the membrane the field decreases rapidly due to dielectric screening by ions and polarized molecules. Thus, the voltage sensing part of an indicator has to be within the membrane to experience any influence exerted by V_m . This effectively reduces the volume from where the activity can be measured to the ‘two-dimensional’ space of the membrane [38]. Hence, compared with the bulk cytosol loading of Ca^{2+} indicators, the maximum number of molecules optically reporting changes in V_m is severely limited, making optical V_m measurements relatively weak.

CONCLUSION

Epilepsy is ultimately a disorder of neuronal connections. The development of epilepsy (epileptogenesis), and the generation of spontaneous seizures (ictogenesis), are emergent properties of aberrant functional and structural connections between individual neurons to produce hypersynchrony, and large brain areas to permit propagation and the manifestation of clinical signs and symptoms. Elucidation of the networks responsible for epileptogenesis and ictogenesis at the whole brain and local circuit levels is now possible using techniques of MRI, electrophysiology, and chemical imaging. The new field of connectomics offers an unprecedented opportunity to delineate the structural and functional anatomy of diverse epilepsy conditions. Localizing the abnormalities responsible for epileptogenesis and ictogenesis, as well as behavioral comorbidities, in specific epilepsy syndromes, as well as in individual patients, will inform more effective surgical interventions, as well as future research into fundamental mechanisms, leading to novel therapies.

Acknowledgments

Original work cited in this review by the authors was funded by grants from the NIH: P01 NS02808, R01 NS033310, U01 NS042372, P20 NS080181 (JE); R01 080655, R01 MH097268, R01 A6040060, R01 EB008432, P41 EB015922 (PT); K23 NS044936 (JS); R01 NS071048 (RS); R01 NS65877 (AB); R01 NS030549, R01 NS075429, R01 MH076994, R21 MH092647 (IM); as well as CURE, the Epilepsy Foundation, and the Epilepsy Therapy Project.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 232).

- 1▪▪ Sporns, O. Discovering the human connectome. Boston: MIT Press; 2012. p. 240Recent textbook on connectomics
- 2▪▪ Richardson MP. Large scale brain models of epilepsy: dynamics meets connectomics. *J Neurol Neurosurg Psychiatry*. 2012; 83:1238–1248. A review of connectomics that considers epilepsy. [PubMed: 22917671]
3. Basser PJ, Mattiello J, LeBihan D. Estimation of the effective self-diffusion tensor from the NMR spin echo. *J Magn Reson B*. 1994; 103:247–254. [PubMed: 8019776]
4. Stejskal EO, Tanner JE. Spin diffusion measurements: spin echoes in the presence of a time-dependent field gradient. *J Chem Phys*. 1965; 42:288–292.
5. Vestergaard-Poulsen P, Wegener G, Hansen B, et al. Diffusion-weighted MRI and quantitative biophysical modeling of hippocampal neurite loss in chronic stress. *PLoS One*. 2011; 6:e20653. [PubMed: 21747929]
6. Vollmar C, O'Muircheartaigh J, Symms MR, et al. Altered microstructural connectivity in juvenile myoclonic epilepsy: the missing link. *Neurology*. 2012; 78:1555–1559. [PubMed: 22551729]
7. Vulliemoz S, Vollmar C, Koepp MJ, et al. Connectivity of the supplementary motor area in juvenile myoclonic epilepsy and frontal lobe epilepsy. *Epilepsia*. 2011; 52:507–514. [PubMed: 21054353]
8. Holt RL, Provenzale JM, Veerapandian A, et al. Structural connectivity of the frontal lobe in children with drug-resistant partial epilepsy. *Epilepsy Behav*. 2011; 21:65–70. [PubMed: 21497558]
9. Shepherd TM, Ozarslan E, King MA, et al. Structural insights from high-resolution diffusion tensor imaging and tractography of the isolated rat hippocampus. *Neuroimage*. 2006; 32:1499–1509. [PubMed: 16806988]
10. Li Y, Du H, Xie B, et al. Cerebellum abnormalities in idiopathic generalized epilepsy with generalized tonic-clonic seizures revealed by diffusion tensor imaging. *PLoS One*. 2010; 5:e15219. [PubMed: 21203575]
- 11▪. Otte WM, Dijkhuizen RM, van Meer MP, et al. Characterization of functional and structural integrity in experimental focal epilepsy: reduced network efficiency coincides with white matter changes. *PLoS One*. 2012; 7:e39078. An elegant study using both functional and structural neuroimaging to determine the anatomical basis of functional network disturbances in epilepsy. [PubMed: 22808026]
12. Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. *NeuroImage*. 2010; 52:1059–1069. [PubMed: 19819337]
13. Daianu, M.; Jahanshad, N.; Nir, TM., et al. Wang, L.; Yushkevich, P.; Sourselin, editors. Analyzing the structural k-core of brain connectivity networks in normal aging and Alzheimer's disease; MICCAI 2012 Workshop on Novel Imaging Biomarkers for Alzheimer's Disease and Related Disorders (NIBAD'12). 2012. p. 52Available at <http://picsl.upenn.edu/Miccai2012/program>
- 14▪. Dennis EL, Jahanshad N, Toga AW, et al. Development of brain structural connectivity between ages 12 and 30: a 4-Tesla diffusion imaging study in 439 adolescents and adults. *NeuroImage*. 2013; 64:671–684. This study defines developmental norms for a variety of brain network metrics in children and young adults, making deviations from normal easier to define and detect. [PubMed: 22982357]

15. Bonilha L, Nesland T, Martz GU, et al. Medial temporal lobe epilepsy is associated with neuronal fibre loss and paradoxical increase in structural connectivity of limbic structures. *J Neurol Neurosurg Psychiatry*. 2012; 83:903–909. [PubMed: 22764263]
16. Calhoun VD, Adali T, Pekar JJ. A method for comparing group fMRI data using independent component analysis: application to visual, motor, and visuomotor tasks. *Mag Reson Imag*. 2004; 22:1181–1191.
17. Pereira FRS, Alessio A, Sercheli MS, et al. Asymmetrical hippocampal connectivity in mesial temporal lobe epilepsy: evidence from resting state fMRI. *BMC Neurosci*. 2010; 11:66. [PubMed: 20525202]
18. Pittau F, Grova C, Moeller F, et al. Patterns of altered functional connectivity in mesial temporal lobe epilepsy. *Epilepsia*. 2012; 53:1013–1023. Investigation of MTLE with seeds in the amygdalae and hippocampi in 23 patients and 23 controls. [PubMed: 22578020]
19. Haneef Z, Lenartowicz A, Yeh HJ, et al. Effect of lateralized temporal lobe epilepsy on the default mode network. *Epilepsy Behav*. 2012; 25:350–357. Investigation of MTLE with seeds in the anterior and posterior default mode network of 23 patients and 13 controls. [PubMed: 23103309]
20. Bettus G, Bartolomei F, Confort-Gouny S, et al. Role of resting state functional connectivity MRI in presurgical investigation of mesial temporal lobe epilepsy. *J Neurol Neurosurg Psychiatry*. 2010; 81:1147–1154. [PubMed: 20547611]
21. Voets NL, Beckmann CF, Cole DM, et al. Structural substrates for resting network disruption in temporal lobe epilepsy. *Brain*. 2012; 135:2350–2357. [PubMed: 22669081]
22. Boucousis SM, Beers CA, Cunningham CJ, et al. Feasibility of an intracranial EEG-fMRI protocol at 3T: Risk assessment and image quality. *NeuroImage*. 2012; 63:1237–1248. [PubMed: 22902923]
23. Carmichael DW, Vulliemmoz S, Rodionov R, et al. Simultaneous intracranial EEG-fMRI in humans: protocol considerations and data quality. *NeuroImage*. 2012; 63:301–309. [PubMed: 22652020]
24. Cunningham CB, Goodyear BG, Badawy R, et al. Intracranial EEG-fMRI analysis of focal epileptiform discharges in humans. *Epilepsia*. 2012; 53:1636–1648. This study defines developmental norms for a variety of brain network metrics in children and young adults, making deviations from normal easier to define and detect. [PubMed: 22881457]
25. Vulliemmoz S, Carmichael DW, Rosenkranz K, et al. Simultaneous intracranial EEG and fMRI of interictal epileptic discharges in humans. *NeuroImage*. 2011; 54:182–190. [PubMed: 20708083]
26. Bartolomei F, Chauvel P, Wendling F. Epileptogenicity of brain structures in human temporal lobe epilepsy: a quantified study from intracerebral EEG. *Brain*. 2008; 131:1818–1830. [PubMed: 18556663]
27. Wendling F, Chauvel P, Biraben A, Bartolomei F. From intracerebral EEG signals to brain connectivity: identification of epileptogenic networks in partial epilepsy. *Front Syst Neurosci*. 2010; 4:154. [PubMed: 21152345]
28. Jung YJ, Kang HC, Choi KO, et al. Localization of ictal onset zones in Lennox-Gastaut syndrome using directional connectivity analysis of intracranial electroencephalography. *Seizure*. 2011; 20:449–457. [PubMed: 21515079]
29. van Mierlo P, Carrette E, Hallez H, et al. Accurate epileptogenic focus localization through time-variant functional connectivity analysis of intracranial electroencephalographic signals. *NeuroImage*. 2011; 56:1122–1133. [PubMed: 21316472]
30. Wilke C, van Drongelen W, Kohrman M, He B. Neocortical seizure foci localization by means of a directed transfer function method. *Epilepsia*. 2010; 51:564–572. [PubMed: 19817817]
31. Bettus G, Ranjeva JP, Wendling F, et al. Interictal functional connectivity of human epileptic networks assessed by intracerebral EEG and BOLD signal fluctuations. *PLoS One*. 2011; 6:e20071. [PubMed: 21625517]
32. Canuet L, Ishii R, Pascual-Marqui RD, et al. Resting-state EEG source localization and functional connectivity in schizophrenia-like psychosis of epilepsy. *PloS One*. 2011; 6:e27863. [PubMed: 22125634]

33. Clemens B, Puskas S, Bessenyei M, et al. EEG functional connectivity of the intrahemispheric cortico-cortical network of idiopathic generalized epilepsy. *Epilepsy Res.* 2011; 96:11–23. [PubMed: 21601428]
34. Horstmann MT, Bialonski S, Noennig N, et al. State dependent properties of epileptic brain networks: comparative graph-theoretical analyses of simultaneously recorded EEG and MEG. *Clin Neurophysiol.* 2010; 121:172–185. [PubMed: 20045375]
35. Bernhardt BC, Chen Z, He Y, et al. Graph-theoretical analysis reveals disrupted small-world organization of cortical thickness correlation networks in temporal lobe epilepsy. *Cereb Cortex.* 2011; 21:2147–2157. [PubMed: 21330467]
36. Terry JR, Benjamin O, Richardson MP. Seizure generation: the role of nodes and networks. *Epilepsia.* 2012; 53:e166–e169. [PubMed: 22709380]
37. Buzsaki G. Large-scale recording of neuronal ensembles. *Nat Neurosci.* 2004; 7:446–451. [PubMed: 15114356]
38. Einevoll GT, Franke F, Hagen E, et al. Towards reliable spike-train recordings from thousands of neurons with multielectrodes. *Curr Opin Neurobiol.* 2012; 22:11–17. [PubMed: 22023727]
39. Peterka DS, Takahashi H, Yuste R. Imaging voltage in neurons. *Neuron.* 2011; 69:9–21. [PubMed: 21220095]
40. Desai M, Kahn I, Knoblich U, et al. Mapping brain networks in awake mice using combined optical neural control and fMRI. *J Neurophysiol.* 2011; 105:1393–1405. [PubMed: 21160013]
41. Lee JH, Durand R, Gradinaru V, et al. Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. *Nature.* 2010; 465:788–792. [PubMed: 20473285]
42. Heim N, Garaschuk O, Friedrich MW, et al. Improved calcium imaging in transgenic mice expressing a troponin C-based biosensor. *Nat Methods.* 2007; 4:127–129. [PubMed: 17259991]
43. Looger LL. Running in reverse: rhodopsins sense voltage. *Nat Methods.* 2012; 9:43–44. [PubMed: 22205516]
44. Looger LL, Griesbeck O. Genetically encoded neural activity indicators. *Curr Opin Neurobiol.* 2012; 22:18–23. [PubMed: 22104761]
45. Heim N, Griesbeck O. Genetically encoded indicators of cellular calcium dynamics based on troponin C and green fluorescent protein. *J Biol Chem.* 2004; 279:14280–14286. [PubMed: 14742421]
46. Knöpfel T, Díez-García J, Akemann W. Optical probing of neuronal circuit dynamics: genetically encoded versus classical fluorescent sensors. *Trends Neurosci.* 2006; 29:160–166. [PubMed: 16443289]
47. Mank M, Reiff DF, Heim N, et al. A FRET-based calcium biosensor with fast signal kinetics and high fluorescence change. *Biophys J.* 2006; 90:1790–1796. [PubMed: 16339891]
48. Mank M, Griesbeck O. Genetically encoded calcium indicators. *Chem Rev.* 2008; 108:1550–1564. [PubMed: 18447377]
49. Baker BJ, Kosmidis EK, Vucinic D, et al. Imaging brain activity with voltage- and calcium-sensitive dyes. *Cell Mol Neurobiol.* 2005; 25:245–282. [PubMed: 16050036]
50. Homma R, Baker BJ, Jin L, et al. Wide-field and two-photon imaging of brain activity with voltage- and calcium-sensitive dyes. *Philos Trans R Soc Lond B Biol Sci.* 2009; 364:2453–2467. [PubMed: 19651647]
51. Mank M, Santos AF, Drenth S, et al. A genetically encoded calcium indicator for chronic in vivo two-photon imaging. *Nat Methods.* 2008; 5:805–811. [PubMed: 19160515]
52. Mao BQ, ei-Sichani F, Aronov D, et al. Dynamics of spontaneous activity in neocortical slices. *Neuron.* 2001; 32:883–898. [PubMed: 11738033]
53. Muri R, Knöpfel T. Activity induced elevations of intracellular calcium concentration in neurons of the deep cerebellar nuclei. *J Neurophysiol.* 1994; 71:420–428. [PubMed: 8158239]
54. Pologruto TA, Yasuda R, Svoboda K. Monitoring neural activity and [Ca²⁺] with genetically encoded Ca²⁺ indicators. *J Neurosci.* 2004; 24:9572–9579. [PubMed: 15509744]

KEY POINTS

- Epileptogenesis and ictogenesis are emergent properties of aberrant neuronal connections.
- MRI diffusion imaging that delineates white matter tracts, characterizing connectivity patterns over large areas of the whole brain, has identified abnormalities associated with several epilepsy syndromes.
- Resting fMRI approaches that delineate patterns of functional connectivity in the whole brain have also identified disturbances associated with epilepsy.
- EEG, alone and in association with structural and fMRI, provides further opportunities to define the abnormal patterns of connectivity that characterize the epilepsy condition and the generation of seizures.
- Electrophysiological and chemical approaches in experimental animals provide novel opportunities to characterize connectivity disturbances at the local circuit level.

FIGURE 1.

Mapping brain connectivity with diffusion MRI. Diffusion-weighted MRI can reveal the profile of water diffusion in brain tissue (colored shapes, top left). Neural pathways can be tracked by following the dominant directions of diffusion in the scan (top right). Whole-brain tractography extracts fibers throughout the brain (row 2), and these may be grouped into bundles that intersect specific regions of interest (such as the cortical regions shown in color in 'Parcellation', row 3). By measuring the density or integrity of fibers that run

between all pairs of cortical regions, a connectivity matrix may be built. The major connections may be shown as a graph or network (row 4).

FIGURE 2.

Functional connectivity of 16 controls based on functional MRI, with a 4-mm seed in left hippocampus. Statistic images are thresholded at Z more than 2.3, with a cluster significance threshold of $P < 0.05$.

FIGURE 3.

Comparison of the resolutions possible with genetically encoded Ca^{2+} -indicators and opto-fMRI. (a) Sagittal brain section and (b) layer V of the motor cortex showing the high resolution fluorescent staining for the GECI CerTn-L15 expressed in Thy-1 transgenic mice (scale bar 0.5 mm in a, and 20 μm in b; adapted with permission from Supplementary Fig. 2 of Heim *et al.* [42]. (c)

Opto-fMRI blood oxygenation level dependent signal (boxcar correlation coloring) in a Thy1-ChR2 expressing mouse under 0.5% isoflurane anesthesia at 100 $\mu\text{m} \times 100 \mu\text{m} \times 500 \mu\text{m}$ resolution during a 10 mW laser power illumination. (Scale bar: 1 mm; blue triangle indicates the site of illumination); adapted with permission from Supplementary Fig. 2 of Desai *et al.* [40]. fMRI, functional MRI; GECI, genetically encoded Ca^{2+} -indicators.