13ª. ESCOLA LATINO-AMERICANA DE VERÃO EM EPILEPSIA
13ª. ESCUELA LATINO-AMERICANA DE VERANO EN EPILEPSIA
13th. LATIN-AMERICAN SUMMER SCHOOL ON EPILEPSY
(LASSE)

SÃO PAULO, BRASIL 7 – 15 DE Março DE 2019
Centro de Convenções Santa Mônica

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LASSE XIII – EPILEPSIA COMO UMA DOENÇA DE REDES NEURAIS

A 13ª Escola Latino-Americana de Verão em Epilepsia (Lasse) é uma atividade educacional da International League against Epilepsy (Ilae) e da Academia Latino-Americana de Epilepsia (Alade) com o apoio da Liga Brasileira de Epilepsia (LBE).

Com início em 2002, as Escolas de verão em epilepsia, organizadas pela Ilae, tornaram-se referência como experiência didática. Como professores e alunos permanecem em contato próximo por cerca de dez dias consecutivos, esse tipo de Escola tem facilitado a integração entre pesquisadores básicos, clínicos, cirurgiões na área de epilepsia e alunos, permitindo uma melhor compreensão das novas descobertas para o benefício das pessoas com epilepsia.

A 13ª Escola Latino-Americana de Verão em Epilepsia (Lasse) a ser realizada em Guarulhos, entre 7 e 15 de março de 2019, abordará o tema Redes neurais em epilepsia _ Da conectividade ao conectoma, uma mudança de paradigmas cuja abordagem é necessária à atualização de jovens epileptologistas latino-americanos.

Agradecemos aos professores e tutores que, de forma tão generosa, abandonam seus afazeres e oferecem-se seu tempo e damos boas-vindas aos alunos da LASSE XIII, razão maior do nosso trabalho.

A COMISSÃO ORGANIZADORA

13th. Latin-American Summer School on Epilepsy (LASSE IX)
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WELCOME AND INTRODUCTION TO LASSE

MARINA BENTIVOGLIO (ITALY)

FROM STRUCTURAL BRAIN CONNECTIVITY TO THE CONNECTOME: PARADIGM SHIFTS IN APPROACHES AND CONCEPTS

(Part of the article *Methods for analysis of brain connectivity: an IFCN-sponsored review* submitted to Clinical Neurophysiology by *Rossini PM, Di Iorio, Bentivoglio M, Bertini G, Ferreri F, Gerloff C, Ilmoniemi RJ, Miraglia F, Nitsche MA, Pestilli F, Rosanova M, Shirota Y, Tesoriero C, Ugawa Y, Vecchio, Ziemann U, Hallett M)*

Structural brain connectivity: experimental approaches and *in vivo* studies of the human brain

(by Marina Bentivoglio, Chiara Tesoriero, Giuseppe Bertini)

Chasing neuronal circuits: a never-ending story

Over the centuries, many paradigm shifts have occurred in the views on neuronal connections, their behavioral output and their alterations in diseases (Bentivoglio and Mazzarello, 2010). The "neuron doctrine", which extended cell theory to the nervous system, was enunciated in 1891 (Shepherd, 2015). A breakthrough in the visualization of neurons was provided by the "black reaction", the metallic impregnation introduced in 1873 by Camillo Golgi (1843-1926). Golgi staining revealed neurons, including their processes, in their entirety and with unprecedented detail. This allowed studies of neuronal circuits (Golgi, 1885), and still allows the investigations of the local neuronal circuitry of randomly impregnated neurons (**Fig. 1A**), also in tissue blocks of *post-mortem* human brain. The revelation power of the Golgi method is only matched after more than one century by genetic cell tagging with fluorescent proteins, or intracellular neuron filling (e.g., in surgically resected tissue blocks of the human brain) (**Fig. 1B,C**).

The champion of the "neuron doctrine" was Santiago Ramón y Cajal (1852–1934), who accomplished a monumental work, largely based on the Golgi stain, in which he provided a map of neuronal connectivity in the mammalian brain (Cajal, 1909, 1995). The debate between Cajal and Golgi—who had adhered to the reticular theory of nervous system organization—boosted neuroscience studies, focusing interest on the gray matter. White matter investigations were essentially descriptive, based on manual dissections and on the study of brain sections with the myelin stain introduced by Carl Weigert (1845–1904). Seminal contributions on the organization of fiber bundles in the human brain were provided by Carl Wernicke (1848–1900) and Joseph Jules Déjérine (1849–1917) (Schmahmann and Pandya, 2007).

The second half of the twentieth century witnessed a revolution in the experimental studies of neuronal connections, together with the explosion of neuroscience in the last decades of the century. As briefly discussed below, novel powerful techniques were introduced. The exploration of connectivity in the human brain remained, however, a challenging problem until the introduction of *in vivo* imaging.

Long-range neuronal connectivity

Anterograde and retrograde degeneration techniques

Pioneering early studies revealed that retrograde degeneration ("secondary atrophy") of neuronal cell bodies and anterograde degeneration of fibers can provide effective tools to trace neuronal connections (Bentivoglio and Mazzarello, 2010) (**Fig. 2A**). Towards the end of the nineteenth century, neuronal alterations consequent to retrograde damage could be assessed by the cell stain (with thionin or toluidine blue) introduced in 1884 by Franz Nissl (1860–1919). Especially influential was the observation of anterograde degeneration of nerve fibers after transection reported in 1851 by Auguste Volney Waller (1816–1870) and named after him "Wallerian degeneration" (**Fig. 2A**).

Besides its implications for the trophic dependence of the axon from the cell body, this finding paved the way to the introduction of anterograde tract tracing methods based on silver impregnation of degenerating fibers after experimental lesions (Nauta and Gygax, 1951; Fink and Heimer, 1967). Metal impregnation stains are capricious and laborious, and degeneration methods have limited sensitivity, but these techniques gave a great impulse to experimental neuroanatomical studies. Importantly, anterograde degeneration revealed by modifications of silver impregnation was also applied to *post-mortem* investigations on the human brain, especially after restricted lesions occurring a few weeks before death (Mesulam, 1979).

Classical experimental tract tracing techniques based on axonal transport

A turning point in the study of structural brain connectivity was the discovery of anterograde and retrograde axonal transport (Bentivoglio, 1999). Axonal transport requires live axons; the active transport of tracers obviously cannot be applied to the human brain. Findings obtained with tract tracing based on axonal transport represent nowadays the "ground truth" for studies of the human brain based on *in vivo* imaging, and in particular on diffusion tractography.

Anterograde tract tracing based on the use of tritiated amino acids revealed by autoradiography was introduced in the early 1970s (Cowan et al., 1972). With this approach, trajectories and terminal fields of fibers originating from the tracer injection site (**Fig. 3**) could be delineated in detail. Anterograde tract tracing approaches have then been implemented (Gerfen and Sawchenko, 1984; Glover et al., 1986). In the same years, the discovery of retrograde axonal transport (Kristensson, 1970; Kristensson and Olsson, 1971) introduced as a tool the enzyme horseradish peroxidase (HRP), visualized by a histochemical reaction, which was soon applied to experimental retrograde tracing of the origin of projections to the tracer injection site (LaVail and LaVail, 1972) (**Fig. 3**).

The introduction of other retrograde tracers rapidly followed to increase sensitivity, combine tracers for multiple retrograde labeling for the study of branched connections, combine retrograde tracing with immunohistochemistry or in situ hybridization for the neurochemical characterization of pathways, and so forth. Fluorescent retrograde tracers turned out to be especially effective and versatile for these applications (e.g. Bentivoglio et al., 1980; Kuypers et al., 1980; Schmued and Fallon, 1986).

Conventional tract tracing has been implemented in recent years with genetic tracing for the study of the connectivity of specific neurons using cell-type-specific promoters (Oh et al., 2009). Most anterograde and retrograde tracers explore monosynaptic connections since they can cross synapses only in minute amounts, ineffective for transsynaptic tracing unless a bolus is injected, which is not feasible in the brain. Neurotropic viruses, which travel through axons and replicate in infected neurons, can instead provide tracing tools (Kristensson et al., 1974) applicable to trans-synaptic tract tracing (Kuypers and Ugolini, 1990) thanks to their propagation across synapses (**Fig. 3**).

Novel approaches to experimental tract tracing: optogenetics and chemogenetics

These innovative techniques are increasingly used to investigate the relationship between neuronal activity, neuronal circuits, and behavior.

The term optogenetics was introduced in 2006 (Deisseroth et al., 2006) referring to the general optogenetic discovery (Boyden et al., 2005). By combining genetic and optical methods, optogenetics utilizes molecular light-sensors to switch on and off neuronal electrical activity. Optogenetics thus allow to investigate neurons and neuronal circuits underlying specific behaviors at the time scale of milliseconds. By this approach, functional effects of defined neuronal cell types can be controlled in living tissue and in freely moving animals (Deisseroth, 2015). Optogenetics has also been combined with functional MRI for the experimental study of cell-type-specific contributions to behavioral output together with a “whole brain read-out” at the millimeter scale (Lee et al., 2017). From the translational point of view, applications of optogenetics in humans for therapeutic purposes are currently envisaged. Clinical applications of the optogenetic system will require obvious implementation and cross-disciplinary know-how (Delbeke et al., 2017).

The term "chemogenetics" was used to describe experiments of site-specific functional group modifications for the analysis of DNA-protein interactions (Strobel, 1998). Currently, the term is used to indicate the processes by which "designer macromolecules" interact with previously unrecognized small molecules (Roth, 2016). Over the past two decades, chemogenetically engineered molecules (kinases, non-kinase enzymes, G protein-coupled receptors, ligand-gated ion channels) have been used experimentally for cell-specific targeting; these molecules modulate cell signaling, turning neuronal circuits on and off. Among chemogenetically engineered protein classes, the most commonly used are the so-called Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) (Roth, 2016).

Local neurocircuitry in the human brain

Diffusion of dyes

An attempt to trace connections in the human brain using in vitro diffusion of wheat germ agglutinin conjugated with HRP gave very limited results (Haber, 1988). More interesting findings were obtained using the diffusion of lipophilic dyes along cell membranes in fixed tissue blocks (**Fig. 2E**). The fluorescent dyes carbocyanines, and in particular DiI and DiO (Honig and Hume, 1989) proved useful for this application. However, dye diffusion can label axons only for a few millimeters, requiring a tracing time of several weeks. Other dyes have been introduced (Heilingoetter and Jensen, 2016), and in particular NeuroVue dyes, which can trace axons for slightly longer distances and at faster diffusion rates than carbocyanines (Fritzsch et al., 2005). The limitations of *ex vivo* tracing, however, hamper its application for extensive fiber tracking in the human brain.

Seeing through: tissue clarification

The natural 3D structure of cells – especially neurons and glial cells, which extend their ramifications in many directions – requires volumetric imaging. The heterogeneous chemical composition of biological tissues (mostly water, proteins, and lipids) generates substantial scattering of the transmitted light, especially at the interface between aqueous protoplasm and membrane lipids, thereby hindering microscopic observation of histological sections beyond a certain thickness. Replacing lipids with a medium characterized by the same refractive index as proteins can effectively render tissues transparent while preserving the native molecular profile and tissue structure, allowing the microscopic observation of the microcircuitry of labeled (e.g., by immunohistochemistry or fluorescent protein tagging) elements.

Aqueous-based clearing techniques are currently widely used and are based on the reduction of light scattering by immersion in a high-refractive-index molecule solution. A breakthrough has been provided by a brain-hydrogel hybrid formed by the so-called CLARITY (Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging / immunostaining / in situ hybridization-compatible Tissue Hydrogel) (Chung et al., 2013). The clarification of thick tissue blocks, such as those useful for the study of the human brain (**Fig. 1D**) remains, however, a challenge. A method to adapt CLARITY to human brain samples with a thickness up to 8 mm has been recently proposed (Morawski et al., 2018). Of note, bridging historical and modern approaches to microcircuits, the Golgi (Golgi-Cox) stain is currently optimized for the use with CLARITY approaches, and could be useful for the study of microcircuitry and the comparison with microstructure MRI data (Kassem et al., 2017).

Diffusion tractography

Diffusion-weighted imaging (DWI), a computational reconstruction method of diffusion-weighted MR images (tractography), allows quantitative estimates *in vivo* of the organization of fiber bundles (tractograms). The characteristic color coding of reconstructed fiber bundles results in images attractive also to the public at large, thus making this approach a very popular insight in the human

brain. (*This method is extensively presented in another part of the review from which this text has been extracted*).

The diffusion coefficient measures the ease of the translational motion of water in tissues. Main DWI acquisition schemes are provided by diffusion tensor imaging (DTI) (**Fig. 2B**), diffusion spectrum imaging (DSI), and high angular resolution diffusion imaging (HARDI). DTI utilizes a tensor model (a matrix of measured diffusion in three orthogonal planes) to characterize the water diffusion properties through myelinated nerve fibers (Basser et al., 1994). Fiber orientation profiles derive from the statistical profile of the displacement of water molecules at a voxel scale and fiber trajectories are inferred from adjacent similar diffusion profiles (Thomas et al., 2014). DSI adds to DTI the capability of resolving multiple directions in each voxel (Wedeen et al., 2005), thus improving also the tracking of intersecting fibers. HARDI improves the accuracy of tractography by using a large number of diffusion-encoding gradients with a reasonable scanning time.

After the first validation study in the macaque brain (Parker et al., 2002), a number of validation studies have been performed, with rather positive or more critical conclusions. For example, the comparison of DSI in the light of extensive autoradiographic tract tracing data on long association pathways in the monkey cerebral hemispheres was found to replicate main features of these fiber tracts (Schmahmann et al., 2007). This comparison proved useful and effective for major cortical fiber bundles (superior, middle and inferior longitudinal fasciculi, fronto-occipital fasciculus, uncinate and arcuate fasciculi, cingulum bundle) (Schmahmann et al., 2007). Another study, based on DWI approaches to the monkey brain, reached more critical conclusions on the potential for accurate fiber tracing (Thomas et al., 2014). The results of a recent "open international tractography challenge", tractograms produced by 20 research groups turned out to contain 90% of the ground truth bundles, but were also reported to "contain many more invalid than valid bundles" (Maier-Hein et al., 2017). These results encourage innovation.

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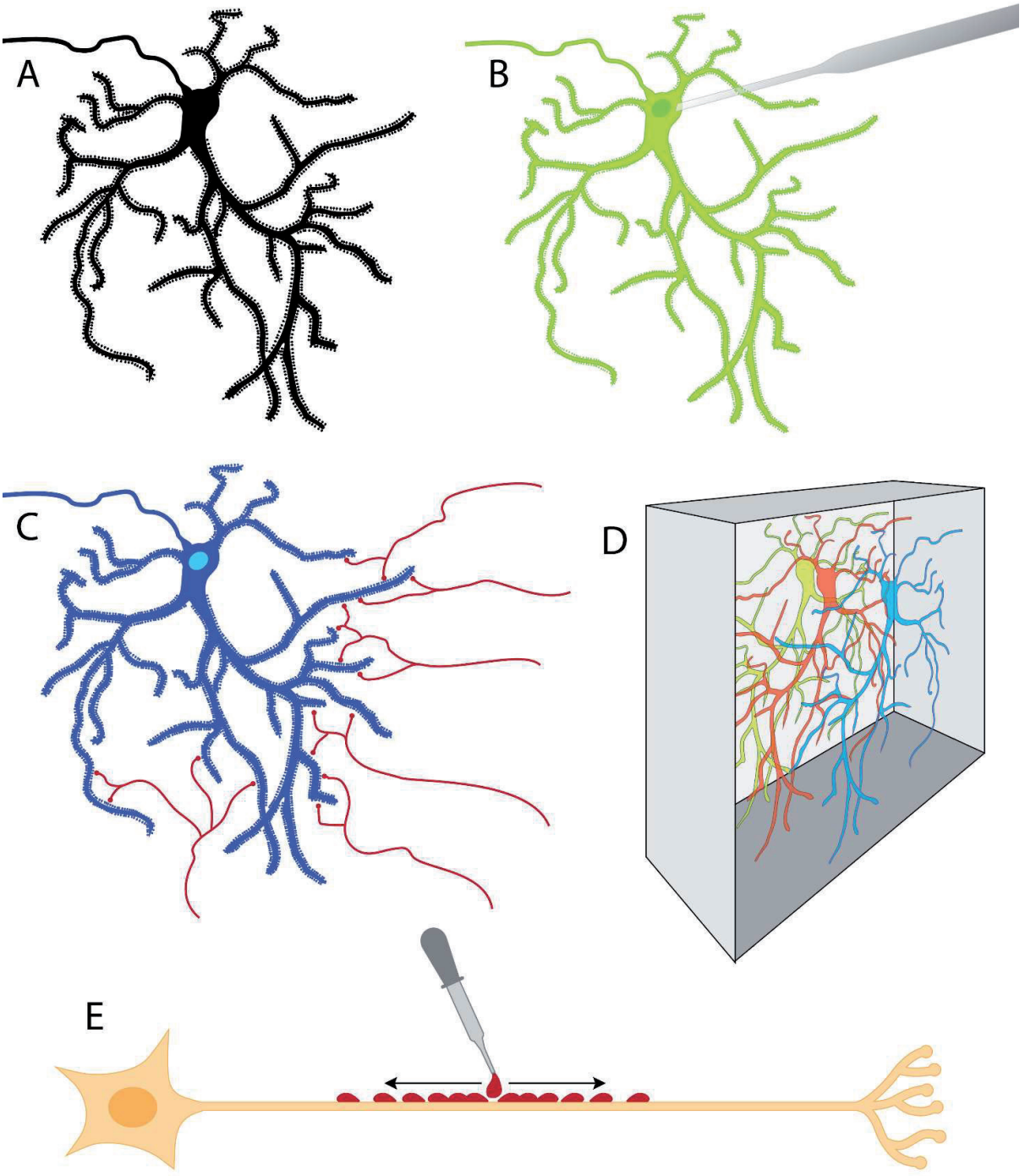

Fig. 1 Histological methods to study local neuronal connectivity and applicable to human brain samples. **A**. The Golgi silver impregnation entirely fills neuronal cell bodies and their processes, allowing detailed visualization and reconstructions; on the other hand, with the Golgi stain it is impossible to predict which cells will be impregnated in any given preparation. **B**. Filling neurons with fluorophores, as part of *in-vitro* electrophysiological experiments (for example in surgically resected brain tissue), allows correlating microscopic morphology with the functional properties of individual neurons. **C**. Immunocytochemistry targets specific cellular markers, and combining different labels allows the study, for example, of connectivity at the individual synapse level. **D**.

Schematic representation of the clarification approach in which brain tissue blocks are rendered transparent and immunocytochemically labeled neurons can be visualized in 3D. **E**. Lipophilic dyes applied on *ex-vivo* samples of nervous tissue are taken up by cell membranes and diffuse to a certain distance, thereby tracing short-range connections, also in human preparations.

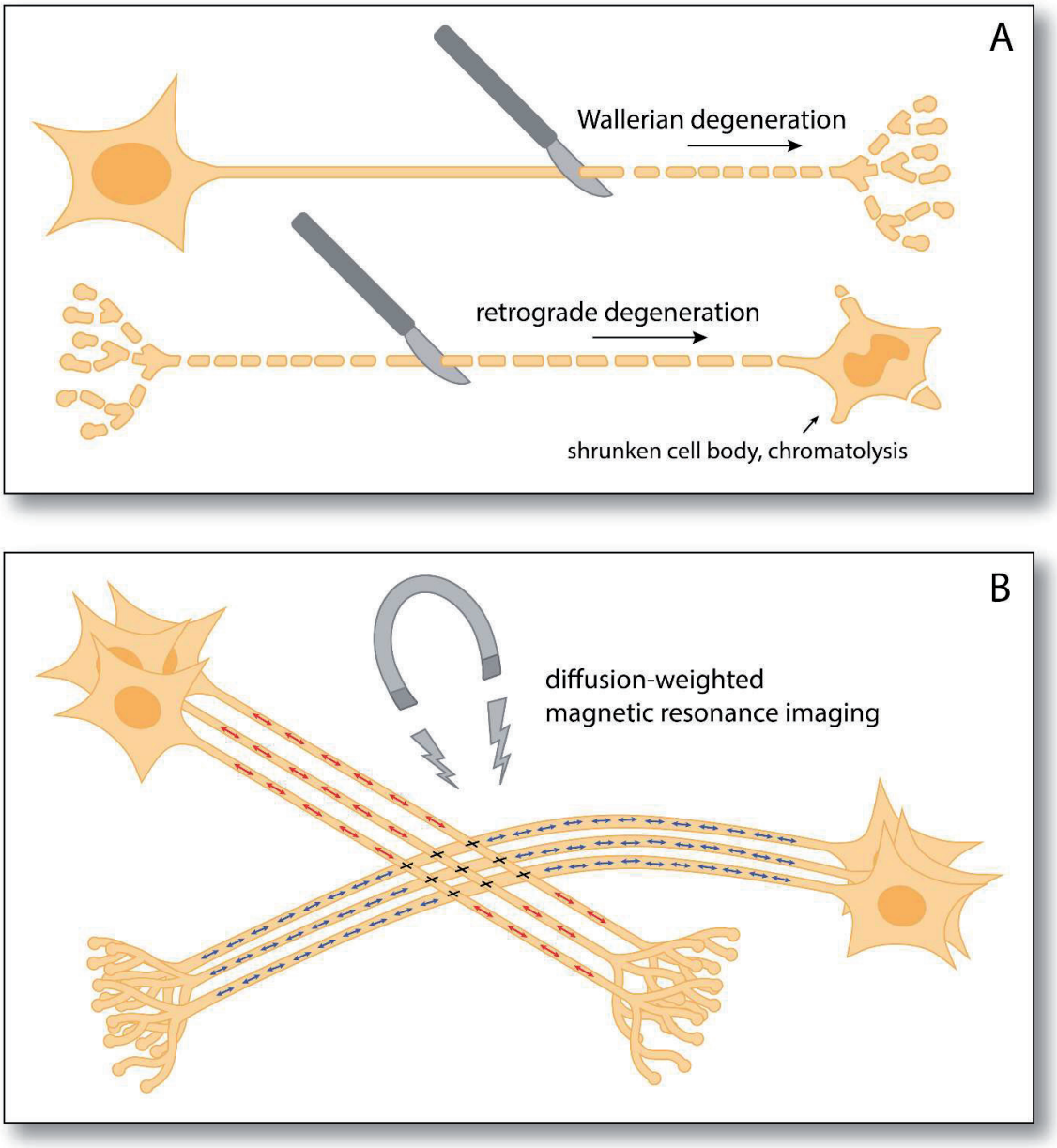

Fig. 2 Methods to study long-range connections in the human brain. **A**. Transections of nerves or CNS fiber bundles invariably cause anterograde (Wallerian) degeneration, i.e. destruction and elimination of the portions of axons and terminal ramifications distal to the lesion; depending on lesion location and size, degeneration can also follow a retrograde path and involve neuronal cell bodies. **B**. Diffusion-weighted magnetic resonance imaging is used to identify *in vivo* the spatial orientation of fiber bundles in the brain, making it possible to reconstruct central pathways. An important limitation of the technique is that in areas where fibers intersect, the signal averages out and accurate directions cannot be established.

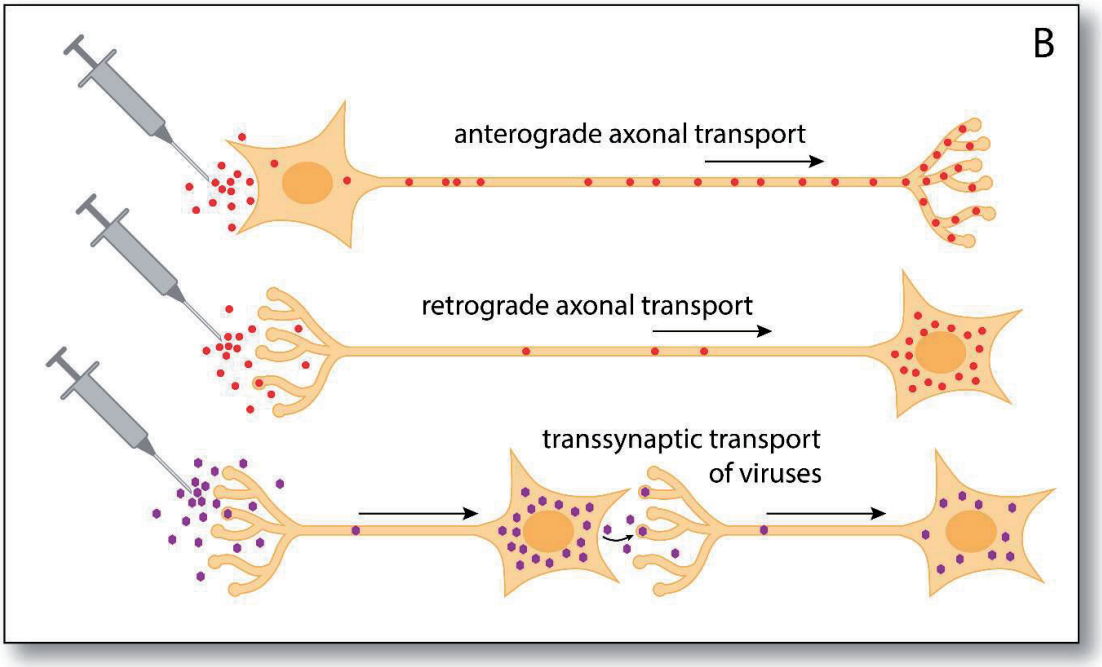

Fig. 3 (added for LASSE Syllabus) Experimental tract-tracing methods based on axonal transport. Axonal transport, the physiological mechanism by which cell bodies exchange cytosolic and membrane-bound components with distant terminals, has been exploited to obtain the most accurate reconstructions of neuronal connections to date. Substances surgically injected into a region of interest are taken up by neurons and are actively transported along axons, thereby acting as tracers. A few days after tracer administration, different visualization techniques allow the precise identification of connections. **Anterograde tracers** enter cell bodies and travel down the axons. Staining reveals fiber bundles and terminal fields. **Retrograde tracers** are taken up by terminals and are carried to the cell body, where they accumulate and produce intense staining of neurons that project to the injected area. Finally, **neurotropic viruses** have been used to trace multisynaptic pathways by exploiting their biological ability to infect peripheral tissues, enter nerve terminals and travel along the fibers, replicate in the cell body, and spread to nearby terminals to repeat the process. Careful determination of time intervals between experimental infection and histological staining of viruses allows to effectively trace synaptic chains.

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Giuseppe Bertini (Italy)

BRAIN WIRING: FROM CIRCUITS TO SYSTEMS

REVIEWS

MEMORY SYSTEMS

The anatomy of memory: an interactive overview of the parahippocampal–hippocampal network

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Abstract | Converging evidence suggests that each parahippocampal and hippocampal subregion contributes uniquely to the encoding, consolidation and retrieval of declarative memories, but their precise roles remain elusive. Current functional thinking does not fully incorporate the intricately connected networks that link these subregions, owing to their organizational complexity; however, such detailed anatomical knowledge is of pivotal importance for comprehending the unique functional contribution of each subregion. We have therefore developed an interactive diagram with the aim to display all of the currently known anatomical connections of the rat parahippocampal–hippocampal network. In this Review, we integrate the existing anatomical knowledge into a concise description of this network and discuss the functional implications of some relatively underexposed connections.

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In the more than 100 years since the first explorations of the parahippocampal–hippocampal network by Ramon y Cajal[1], numerous detailed anatomical tract-tracing analyses (BOX 1) have been published. These studies were sparked by the discovery of a prominent relationship between declarative memory and structures in the human medial temporal lobe, in particular the hippocampal formation (HF)[2]; the importance of the parahippocampal region (PHR) for memory was established only later[3]. An increasingly complex picture of the connectivity within and between the HF and the PHR has emerged over the years, and comprehensive knowledge of the PHR–HF network lies at the basis of understanding its functions[4].

The level of anatomical detail at which an experiment must be carried out or results interpreted depends on the questions under investigation. In some instances, the effects of experimental manipulations can be interpreted using connectivity data at an overall network level (without taking the details of local networks into account). Other studies require more detail, but even those studies that benefit from a detailed understanding of the circuitry often do not, for a variety of reasons, take all the known connections into consideration. Sometimes connections are simply overlooked, whereas other times connections are intentionally left out because they seem to have no function and are therefore considered irrelevant for a particular theoretical interpretation. Eventually, such underexposed connections tend to be erased from the common scientific memory. For this Review, we have assembled the extensive anatomical PHR–HF connectivity literature, focusing on all known connections of one frequently used experimental animal: the rat. We introduce a new approach to describe the network connectivity that uses an interactive diagram to display the complete PHR–HF connectivity (see Supplementary information S1 (figure) and Supplementary information S2 (box)). The complex and detailed connectivity patterns in this diagram are made accessible through the ability to switch on and off individual or groups of network connections between cortical layers and/or anatomical areas. The information this diagram provides could prove to be useful at a time when research is moving beyond the functional explanations that can be provided by a PHR–HF circuitry model that contains only a subset of the connections; moreover, it might lead to a re-evaluation of the functional importance of connections that have previously been ignored.

This Review first describes the anatomical concepts that are essential to understanding the PHR–HF circuitry (for an extensive description, see REFS 5–7). Next, it presents an overview of the main PHR–HF circuits as well as of some of the lesser-known aspects of the circuitry, using the interactive diagram (Supplementary information S1 (figure)). Subsequently, it shows how having detailed knowledge of the PHR–HF circuitry can

Box 1 | **Neuroanatomical tract-tracing methods**

Most of what is known today about the pathways that connect neurons in different brain regions has been discovered by using neuroanatomical tract-tracing techniques[156]. A tracer is a substance that allows such pathways to be visualized. Tracers can be injected intracellularly to label the dendrites and axons of a neuron. Both autofluorescent dyes (for example, Lucifer yellow and Alexa dyes) and biotin-derived dyes are often used for intracellular labelling, as they can be easily visualized using fluorescent microscopy. Alternatively, a tracer can be injected at a stereotaxically defined extracellular location in the *in vivo* brain. The tracer is taken up by neurons at the injection site and is transported or diffuses within cells. A tracer substance can be transported anterogradely from the soma towards the axon terminals (for example, *Phaseolus vulgaris* leucoagglutinin), retrogradely from the axon terminals towards the soma (for example, Fast Blue), or it can be transported in both directions (for example, horseradish peroxidase). Another tract-tracing method involves creating small lesions and visualizing the resulting degeneration; the labelled connections are generally assessed using light microscopy. Electron microscopy can be used to visualize whether a presynaptic axon contacts a postsynaptic element. This is a very accurate but time-consuming method because only small pieces of tissue can be examined at one time. Alternatively, confocal microscopy allows three-dimensional reconstruction of larger pieces of tissue and can indicate whether pre- and postsynaptic elements are likely to form a synapse. A question of current interest is whether confocal microscopy is reliable enough for indicating such contacts. In order to increase our understanding of the connectivity of the brain and its related function, accurate numbers that provide information about pathways' projection intensity and termination density are needed. To achieve this, techniques using viral tracers[157] and new genetic tools[158] are being developed.

aid one's understanding of some of the functional processes that engage the PHR–HF regions, such as memory formation, spatial navigation and temporal dynamics.

Hippocampal–parahippocampal anatomy

The rat HF is a C-shaped structure that is situated in the caudal part of the brain. Three distinct subregions can be distinguished (FIG. 1): the dentate gyrus (DG), the hippocampus proper (consisting of CA3, CA2 and CA1) and the subiculum. The cortex that forms the HF has a three-layered appearance. The first layer is a deep layer, comprising a mixture of afferent and efferent fibres and interneurons. In the DG this layer is called the hilus, whereas in the CA regions it is referred to as the stratum oriens. Superficial to this polymorph layer is the cell layer, which is composed of principal cells and interneurons. In the DG this layer is called the granule layer, whereas in the CA regions and the subiculum it is referred to as the pyramidal cell layer (stratum pyramidale). The most superficial layer is referred to as the molecular layer (the stratum moleculare) in the DG and the subiculum. In the CA region the molecular layer is subdivided into a number of sublayers. In CA3, three sublayers are distinguished: the stratum lucidum, which receives input from the DG; the stratum radiatum, comprising the apical dendrites of the neurons located in the stratum pyramidale; and, most superficially, the stratum lacunosum-moleculare, comprising the apical tufts of the apical dendrites. The lamination in CA2 and CA1 is similar, with the exception that the stratum lucidum is missing.

The PHR lies adjacent to the HF, bordering the subiculum. It is divided into five subregions: the presubiculum, the parasubiculum, the entorhinal cortex (EC, consisting of medial (MEA) and lateral (LEA) areas), the perirhinal cortex (PER, consisting of Brodmann areas (A) 35 and 36) and the postrhinal cortex (POR). The PHR is generally described as having six layers. The coordinate systems that define position within the HF and the PHR are explained in FIG. 1.

Circuitry of the PHR–HF region

In the interactive diagram (FIG. 2; Supplementary information S1 (figure)) we attempted to display all of the PHR–HF connections that have been reported in the anatomical literature concerning the rat (for references see Supplementary information S3 (table)). The interactive diagram contains almost 1,600 connections, which can be displayed at a customizable level of complexity. This allows easy comparisons between the detailed PHR–HF circuitry illustrated by the diagram and a 'standard' model of this circuitry (FIG. 3), which displays the subset of connections that are currently most often used in the field (based on an analysis of a selection of recent key studies[8–15]).

Connectivity within the PHR. In the standard model (FIG. 3), the projections from the PER and the POR to the EC are often depicted with a topology that emphasizes PER-to-LEA and POR-to-MEA relationships. However, as can be seen in the interactive diagram, the available data indicate (see figure 1a in Supplementary information S4 (figure)) that the POR also projects to the LEA, although quantitatively to a lesser extent than the PER (4.9% versus 15.6%, respectively, of the total cortical input)[16]. Likewise, the PER also projects to the MEA (see figure 1b in Supplementary information S4 (figure)), contributing a level of cortical input equal to that of the POR (7.5%)[16]. Neurons in layers II, III, V and VI of A35 and A36 of the PER project in a convergent way to LEA layers II and III[17], whereas the PER projection to the MEA arises mainly from A36 (REFS 16,17). The POR projection to the LEA arises from layers II, III, V and VI and terminates in layers II and III[16,17]. The POR projection to the MEA originates from the same layers and terminates preferentially in the superficial layers, although some fibres can be seen in the deep layers of the MEA[16,18].

The EC reciprocates the projections from the PER and the POR, as depicted in the standard model. A detailed look at the interactive diagram shows that there are projections from layers III and V of the LEA to all layers of A35 and A36 (REFS 16,17,19), and from the MEA to all layers of A35 (REFS 16,17,20) (see figure 2a in Supplementary information S4 (figure)). The MEA also projects to A36 (REFS 16,21). Both the MEA and the LEA project to the POR, but details of the topography of this connection in the rat are currently not available[16,21,22] (see figure 2b in Supplementary information S4 (figure)).

Traditionally, little attention has been paid to the connections between the PER and the POR, although there is extensive connectivity between these regions. POR layers II and V project to all layers of A35 and A36; POR layer III also projects to A36 (see figure 3a in Supplementary information S4 (figure)). Rostral levels of the POR provide the densest projection to caudal levels of A35 and

Temporal dynamics
Properties of neurons in a network, such as precise spike times and firing rates, that facilitate information transfer.

Convergence
When inputs from different brain regions congregate on to single cells or on to a local network in another region.

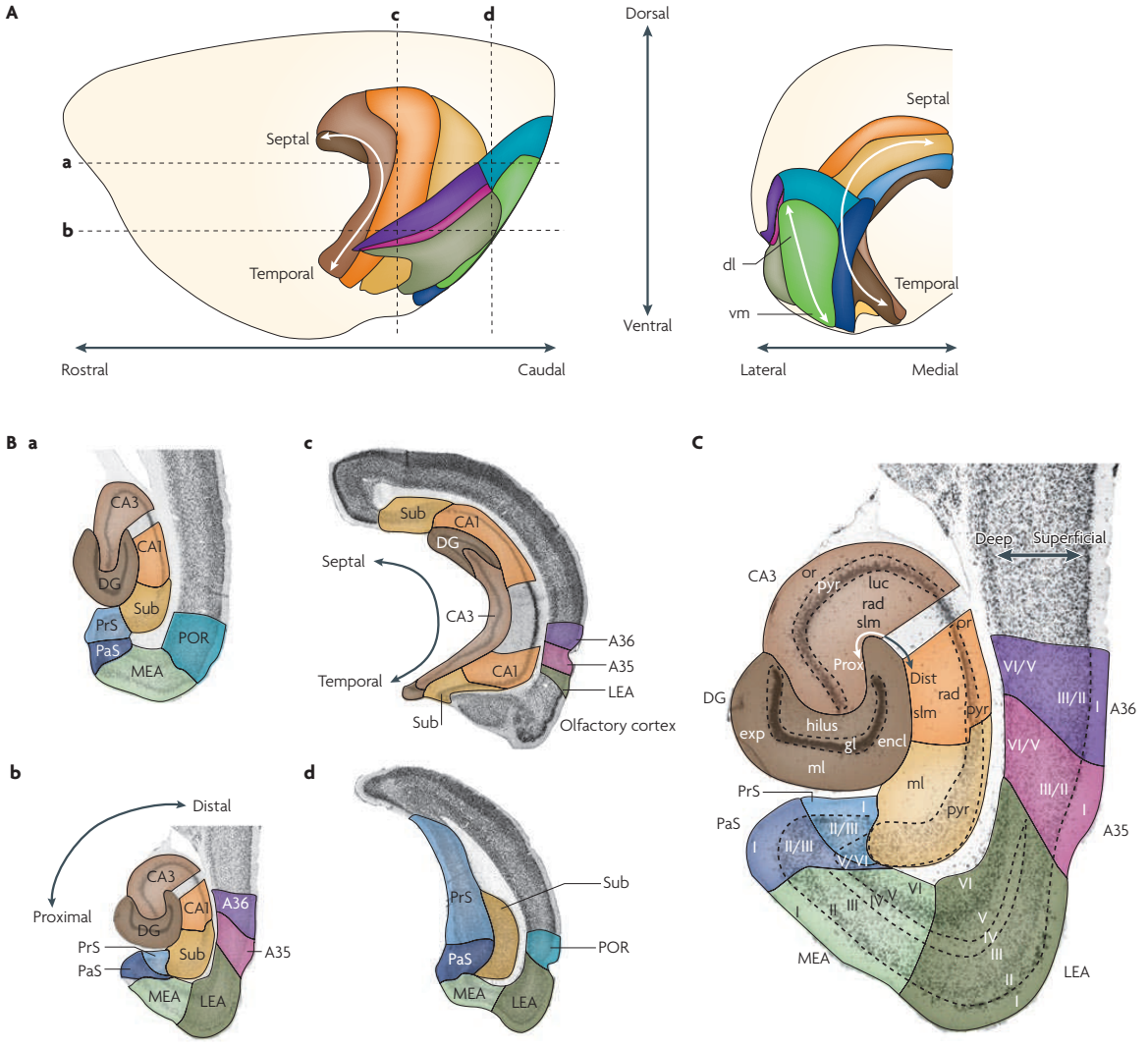

Figure 1 | **Representations of the hippocampal formation and the parahippocampal region in the rat brain.** **A** | Lateral (left panel) and caudal (right panel) views. For orientation in the hippocampal formation (consisting of the dentate gyrus (DG; dark brown), CA3 (medium brown), CA2 (not indicated), CA1 (orange) and the subiculum (Sub; yellow)), three axes are indicated: the long or septotemporal axis (also referred to as the dorsoventral axis); the transverse or proximodistal axis, which runs parallel to the cell layer and starts at the DG; and the radial or superficial-to-deep axis, which is defined as being perpendicular to the transverse axis. In the parahippocampal region (green, blue, pink and purple shaded areas), a similar superficial-to-deep axis is used. Additionally, the presubiculum (PrS; medium blue) and parasubiculum (PaS; dark blue) are described by a septotemporal and proximodistal axis. The entorhinal cortex, which has a lateral (LEA; dark green) and a medial (MEA; light green) aspect, is described by a dorsolateral-to-ventromedial gradient and a rostrocaudal axis. The perirhinal cortex (consisting of Brodmann areas (A) 35 (pink) and 36 (purple)) and the postrhinal cortex (POR; blue-green) share the latter axis with the entorhinal cortex and are additionally defined by a dorsoventral orientation. The dashed lines in the left panel indicate the levels of two horizontal sections (**a**,**b**) and two coronal sections (**c**,**d**), which are shown in part **B**. All subfields of the parahippocampal–hippocampal region are colour-coded in correspondence with the interactive diagram in Supplementary information S1 (figure). A further description of the anatomical features of each subfield is provided in the legend of this supplementary information. **C** | A Nissl-stained horizontal cross section (enlarged from part **Bb**) in which the cortical layers and three-dimensional axes are marked. The Roman numerals indicate cortical layers. CA, cornu ammonis; dist, distal; dl, dorsolateral part of the entorhinal cortex; encl, enclosed blade of the DG; exp, exposed blade of the DG; gl, granule cell layer; luc, stratum lucidum; ml, molecular layer; or, stratum oriens; prox, proximal; pyr, pyramidal cell layer; rad, stratum radiatum; slm, stratum lacunosum-moleculare; vm, ventromedial part of the entorhinal cortex.

Reciprocal connections
Bidirectional, equivalent connections between two areas, networks or neurons.

Perforant pathway
Axons that originate in the superficial layers of the EC and are distributed to all fields of the hippocampus.

A36. Additionally, the POR projection to A36 is stronger than that to A35 (REFS 16,17,23). The PER projection to the POR originates in PER layers II, V and VI[16,17,21,23] (see figure 3b in Supplementary information S4 (figure)). The densest projection connects the rostral PER with the caudal POR[17].

A set of intra-PHR connections that is also underexposed in the standard model is the connections between the EC, the presubiculum and the parasubiculum. The dorsolateral MEA projects to septal levels of the presubiculum and the parasubiculum (see figure 4a in Supplementary information S4 (figure)), whereas the ventromedial MEA projects to the temporal presubiculum and parasubiculum[20,22,24–28] (see figure 4b in Supplementary information S4 (figure)). The LEA also projects to the presubiculum and the parasubiculum, but precise topographical information for this projection is absent[19,20,22,25,29,30]. Both the presubiculum and the parasubiculum send projections to the EC. The septal presubiculum projects to the dorsolateral and intermediate part of the MEA (see figure 5a in Supplementary information S4 (figure)), whereas the temporal presubiculum projects to the ventromedial part of the MEA (see figure 5b in Supplementary information S4 (figure)). The superficial layers of the presubiculum project to the deep layers of the LEA[31] and to layers I, II and III of the MEA[27,32–34]. The deep layers of the presubiculum project to all layers of the MEA and predominantly to the deep layers of the LEA[27,34,35]. A detailed topography for the parasubiculum-to-EC connection has not yet been described, but it is known that all layers of the parasubiculum converge on to layer II of the MEA[21,24,30,32,33,36].

Several other connections have been described that have not been incorporated into the standard model shown in FIG. 3. For example, reciprocal connections between the presubiculum/parasubiculum and the PER/POR have been described[21,23], but details are limited. Other connections, such as the intrinsic connections of the EC, are better anatomically characterized, but they remain outside the scope of most models. For example, the MEA and the LEA are strongly interconnected: cells in layers II, III, V and VI of the MEA project to the superficial layers of the LEA[20,37]; LEA layers II and V project to the superficial layers of the MEA[17,20,29,37], whereas LEA layers III and VI project to superficial and deep layers of the MEA[29,37].

PHR projections to the HF. There is a prominent and topologically arranged circuitry between the PHR and the HF. The EC-to-HF circuitry is known as the perforant pathway (FIG. 3). According to the standard view only EC layer II projects to the entire transverse

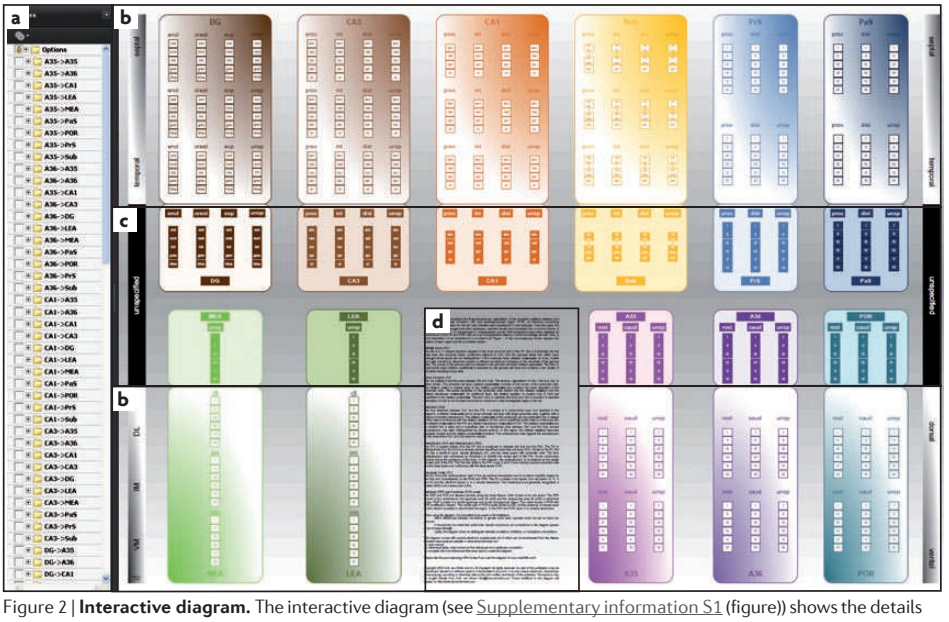

Figure 2 | **Interactive diagram.** The interactive diagram (see Supplementary information S1 (figure)) shows the details of the connectivity in the parahippocampal–hippocampal network, including the topology of the connections. All regions and their three-dimensional axes (for example, the septotemporal axis; see FIG. 1) are included in the diagram. **a** | An alphabetically sorted list of 'from–to' connection groups that can be switched on or off. In front of each group is a + sign. Clicking this expands the list of individual connections that make up the group, allowing one to select connections originating from a specific cortical layer or according to a specific three-dimensional projection pattern (for example, only dorsolateral entorhinal cortex to septal hippocampus connections). **b** | In this area of the diagram the selected connectivity within and between subregions is displayed with full topological detail. **c** | In some cases topological detail is not available; these connections are displayed with a reduced level of topological detail in the centre of the diagram. Connections between diagram elements in parts **b** and **c** also exist. **d** | The diagram legend provides a detailed anatomical description of all subregions. Refer to the diagram manual in Supplementary information S2 (box) for detailed instructions.

Divergence
When one brain region sends projections to several different brain regions.

Mossy fibres
The main projection of DG granular cells to CA3; characterized by high concentrations of zinc.

Schaffer collaterals
The axon collaterals of the CA3 pyramidal cells that project to CA1.

extent of the DG. In fact, EC layers III, V and VI also contribute to this projection, although to a lesser extent. The details of the EC-to-DG[19,22,24–26,38–51] and EC-to-CA3[19,20,22,25,29,38,41,42,44,48–50,52] projections might provide clues to their function. For example, in the molecular layer of the DG and the stratum lacunosum-moleculare of CA3, projections from the EC converge on to the apical dendrites of dentate principal cells and interneurons. Specifically, the LEA projects to the outer third of the molecular layer of the DG, and the MEA projects to the middle third of this layer. A similar pattern of convergence[53] is observed in CA3, where the LEA projection terminates in the superficial part of the stratum lacunosum-moleculare and the MEA projection terminates in the deep part of this layer. In addition to convergence, divergence[53] of the EC projections to the DG and CA3 also occurs, as individual layer II cells project to both the DG and CA3 (REFS 48,54).

The organization of the EC projection to CA1 and the subiculum is markedly different from that of the EC-to-DG or EC-to-CA3 projection. The origin of the main projection from the EC to the stratum lacunosum-moleculare of CA1 and the molecular layer of the subiculum lies in layer III although, again, other layers (II, V and VI) contribute to a lesser extent to this projection[20,22,25,26,29,38,41–43,46,49,51,52,55–57]. Another striking feature of this pathway is the difference between the LEA and MEA projections along the transverse axis. The LEA projects to the distal part of CA1 and the proximal subiculum, whereas the MEA projects to the proximal part of CA1 and the distal subiculum[38,49,52]. This segregation suggests that the input from the LEA and the MEA is processed in different parts of CA1 and the subiculum. This idea is supported by the observation that the segregation of the EC input to CA1 and the subiculum is maintained in the intra-HF projection from CA1 to the subiculum (see next subsection).

In addition to this topology along the transverse axis of the HF, there is a topological organization of connections between the dorsolateral–ventromedial axis of the EC and the longitudinal axis of the HF: the dorsolateral parts of the LEA and the MEA project to the septal HF, the intermediate part of the EC projects to intermediate septotemporal levels, and the ventromedial EC projects to the temporal HF[40,58,59]. According to some reports, the actual organization of the perforant pathway is more widespread (see figure 6 in Supplementary information S4 (figure)), such that this topography relates to the densest projections, whereas weaker components show a more divergent distribution along the septotemporal axis[46,50]. Such a broader projection pattern along the septotemporal axis of the HF may affect information processing.

The EC-to-HF projection forms the main PHR connection to the HF. Other PHR subregions have also been observed to project to the HF directly, although less strongly than the EC and most of them are not included in the standard view. Neurons in all layers of the presubiculum and the parasubiculum project to the stratum moleculare of the DG[32,44,60] and the subiculum[24,32,60,61] and to the stratum lacunosum-moleculare of CA3 (REFS 32,44) and CA1 (REFS 32,44,60) (see figures 7a and 7b in Supplementary information S4 (figure)). Another example of underexposed circuitry is the direct projection from the PER and the POR to the HF. Both A35 and A36 have been reported to project to CA1 and the subiculum[23,62]. The POR has been suggested to project to all sub-areas of the HF[23], but another report indicates only direct projections to CA1 and the subiculum[57].

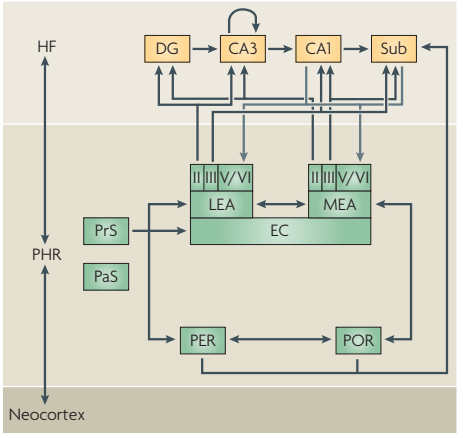

Figure 3 | **The standard view of parahippocampal–hippocampal circuitry.** The standard view that is presented here is based on various circuitry models from recent articles[8–15]. According to this standard view, neocortical projections are aimed at the parahippocampal region (PHR), which in turn provides the main source of input to the hippocampal formation (HF). In the PHR, two parallel projection streams are discerned: the perirhinal cortex (PER) projects to the lateral entorhinal cortex (LEA), and the postrhinal cortex (POR) projects to the medial entorhinal cortex (MEA). The entorhinal cortex (EC) reciprocates the connections from the PER and the POR. Additionally, the EC receives input from the presubiculum (PrS). The EC is the source of the perforant pathway, which projects to all subregions of the hippocampal formation. Entorhinal layer II projects to the dentate gyrus (DG) and CA3, whereas layer III projects to CA1 and the subiculum (Sub). The polysynaptic pathway, an extended version of the traditional trisynaptic pathway, describes a unidirectional route that connects all subregions of the HF sequentially. In short, the DG granule cells give rise to the mossy fibre pathway, which targets CA3. The CA3 Schaffer collaterals project to CA1 and, lastly, CA1 projects to the Sub. Output from the HF arises in CA1 and the Sub and is directed to the PHR, in particular to the deep layers of the EC. The Roman numerals indicate cortical layers.

Connectivity within the HF. In the standard model the first step of the polysynaptic HF pathway (FIG. 3; see also figure 8a in Supplementary information S4 (figure)) is formed by a unidirectional projection from the DG to CA3: the mossy fibres. The Schaffer collaterals, which originate in CA3 and project to CA1, are the next step in the polysynaptic loop. A detailed look at these connections shows an interesting topology along the transverse axis. The distal part of CA3 projects to proximal CA1 and, conversely, the proximal part of CA3 projects to distal

CA1 (REFS 63–65). The topography of the projections that arise from mid-proximodistal portions of CA3 lies between that of these two projection patterns. The last step in the polysynaptic pathway is the projection from CA1 to the subiculum. The proximal part of the CA1 pyramidal cell layer projects to the distal subiculum, whereas the distal CA1 projects to the proximal part of the subiculum[52,66–69].

In contrast to what is depicted in the standard model, there are several backprojections in the HF. Pyramidal cells in CA3 project back to the hilus and the inner molecular layer of the DG[64,70–74], and all septotemporal levels have this backprojection (see figure 8b in Supplementary information S4 (figure)). The strongest backprojection originates in the temporal levels of CA3 and projects to the temporal part of the DG[71]. Again contrasting the standard idea of unidirectionality, a backprojection from CA1 to CA3 has also been reported; this backprojection most likely arises from inhibitory neurons in the stratum radiatum and stratum oriens of CA1 and projects to the same layers in CA3 (REFS 64,66,67,75) (see figure 8b in Supplementary information S4 (figure)). A backprojection from the subiculum to CA1 has also been reported (see figure 8b in Supplementary information S4 (figure)). This backprojection arises from neurons in the stratum pyramidale of the subiculum and projects to all layers of CA1 (REFS 32,76). Currently, it is not known whether this backprojection is of an excitatory or an inhibitory nature.

Recurrent collaterals of the CA3 region[63,64,70–73,77–81] are well acknowledged in the literature (FIG. 3), and they have been described in the other HF subregions as well (see figure 9 in Supplementary information S4 (figure); these intrinsic recurrent networks are less extensive and are also less investigated in terms of their anatomy and function (see the 'Functional implications' section). In the polymorphic layer of the DG, each granule cell establishes contact with the proximal dendrites of several mossy cells, which return excitatory synapses to granule cell dendrites in the molecular layer[47,64,65,70,80,82–85]. CA1 has recurrent loops that are restricted to one septotemporal level[66,69,75,76,79,86]. In the subiculum, principal cells extend axon collaterals to a substantial part of the subiculum that lies ventral to the site of origin; these collaterals terminate on pyramidal cells and interneurons[32,76,87,88].

HF projections to the PHR. The HF output to the PHR arises from CA1 and the subiculum and, according to the standard view, terminates primarily in the deep layers of the EC. In contrast to this view, several authors have reported direct projections from CA1 (REFS 72,75) and the subiculum[32,89,90] to the superficial layers of both the LEA and the MEA.

There are reciprocal connections between the EC and CA1/the subiculum. The CA1–to–EC projection is organized such that the septotemporal axis of the HF is mapped topologically on to the dorsolateral–ventromedial axis of the EC, comparable to the organization of the strongest EC-to-HF projection[52,72,75]. The transverse output organization also mimics the input — that is, the proximal part of CA1 projects to the MEA (see figure 10a in Supplementary information S4 (figure)) and the distal part of CA1 projects to the LEA[49,52] (see figure 10b in Supplementary information S4 (figure)). The subiculum-to-EC projections have a similar topography along the long[89,91] and transverse axes[49,88,89,91,92], although they seem to be less sharply defined. Moreover, along the transverse axis the organization is opposite to that of the CA1-to-EC connections: the proximal subiculum sends a stronger projection to the LEA and the distal subiculum sends a stronger projection to the MEA, again in line with the overall organization of the EC projections to the subiculum.

Although the CA1/subiculum-to-EC projections form the main part of the HF output to the PHR, other connections to the PHR also exist. For example, CA3 (REFS 24,44,72,78), CA1 (REFS 24,31,44,69,75) and the subiculum[24,31,32,88,89,91,92] all project to the presubiculum and the parasubiculum (see figure 11 in Supplementary information S4 (figure)). The projection from the subiculum to the presubiculum is the best described of these. It follows a septotemporal gradient, such that the septal part of the subiculum projects to the septal presubiculum[31,88,89,91] and the temporal part of the subiculum projects to the temporal presubiculum[24,91]. A projection from the subiculum to the parasubiculum exists, but no detailed information about it is known[24,32,89]. Finally, CA1 and the subiculum project to both the PER and the POR, although no detailed information about the organization of this projection is currently available[21,23].

Functional implications

In the preceding section we compared the details of the PHR–HF circuitry to the standard view, highlighting several underexposed connections. To provide a functional perspective on some of these connections, we now discuss them in the context of three topics that have long been associated with the HF: memory formation, navigation and temporal dynamics.

Memory formation. The first example of how increased knowledge of connections in the PHR and the HF might change our views on the memory function of the HF concerns the idea that the HF is the region in which different types of information are associated in memory. By contrast, the EC is generally defined as a simple input–output structure that keeps the incoming information flows separate by way of two parallel pathways (FIG. 3): the PER-to-LEA-to-HF pathway conveys non-spatial information about external stimuli, whereas the POR-to-MEA-to-HF pathway conveys spatial information[18].

However, there are four arguments that support the notion that, rather than being a simple input–output structure, the EC has a role in more complex associations. First, anatomical evidence shows that PER and POR projections to the EC overlap (see the 'Circuitry' section). Second, there is an extensive network in the EC that reciprocally connects the LEA and the MEA[17,20,29,37]. These first two anatomical characteristics suggest that non-spatial information in the LEA and spatial information in the MEA can become associated at the level of the EC, which is supported by the observation that the LEA

is involved in odour–place associations[93]. Third, deep and superficial layers of the EC are also anatomically interconnected[20,26,29,37,94], and this connection is likely to explain the observation that the firing characteristics of cells in all layers of the MEA have a clear correlation across layers during the performance of spatial tasks[95]. Fourth, according to the classical view (FIG. 3), the superficial EC layers are the input layers to the HF, whereas the deep layers receive hippocampally processed information that they convey back to the cortex. However, the anatomical data summarized in this Review show projections from the deep layers of the EC to the HF, consistent with the finding that activation of the deep layers of the EC is sufficient to activate the DG[96]. Additionally, the HF projects to both deep and superficial EC layers (see the 'Circuitry' section).

We therefore propose that the notion that the EC is a simple, laminated input–output structure needs revision: information becomes integrated before it enters the HF. This suggests that both the HF and the EC associate information that is relevant to memory. As the same types of information are processed by the two structures, the question remains how their functions compare. One way to view the distinctive roles of the regions is that the EC holds a more universal memory representation of the associated information, whereas the HF is involved in processing details of this information through processes such as pattern separation and pattern completion. The observation that activity in the HF increases when a person is recalling details from memory supports the proposal that the HF has a role in processing detailed information[97]. The idea that the EC processes information at an earlier and more generic level than the HF (in which detailed information is processed) corresponds to the idea that the EC holds a universal map that is important to the HF in navigation, as discussed below.

Associative networks and, in particular, the auto-associative network of CA3 have been proposed to be essential for encoding and storing episodic memories[98,99]. The recurrent connections in this area can be theoretically arranged into a number of discrete patterns of activation, called stable states or attractors, and the synaptic strengths of the recurrent connections determine the stable states of this network[98]. Incoming information presumably directs the network into one of its stable states, thus enabling pattern completion[100]. Although the CA3 recurrent network is currently thought to be the most elaborate in the HF, CA1, the DG hilus region and the subiculum also contain recurrent collateral networks (see figure 9 in Supplementary information S4 (figure)) and are likely to exhibit computational characteristics comparable to those of the CA3 recurrent network. One striking feature of the CA1 recurrent network that emerges from the diagram is that the recurrent loops are restricted to one septotemporal level (see figure 9 in Supplementary information S4 (figure)). For example, the input to the septal CA1 from CA3 arises from both septal and intermediate levels of CA3, whereas the input to the temporal CA1 arises from the temporal and intermediate CA3. This input is then processed independently in both the septal and the temporal CA1. It would be interesting to know whether there is also regional specificity of CA1 recurrents along the transverse axis, as the MEA and the LEA project preferentially to different proximodistal regions of CA1 (see the 'PHR projections to the HF' subsection). Preliminary data from recordings in the septal CA1 are in line with this idea and show that cells at different transverse positions have different firing characteristics[101] that are related to the type of information provided by the MEA or LEA inputs (spatial and non-spatial, respectively). We propose that CA1 is divided into subdomains along the combination of the septotemporal and proximodistal axes, and that each subdomain independently processes different, specific combinations of information originating from different input areas. In theory, each of these subdomains would thus be able to encode and store unique input patterns, which may be instrumental in discriminating subtle differences in input cues and may aid pattern separation and completion. This prediction awaits further experimental data, such as detailed recordings along both axes in freely behaving animals.

Navigation. Different types of spatial information, discussed below, are represented in the PHR–HF circuitry, and the circuitry may facilitate the exchange of these different types of information in order to make navigation through an environment possible. The same circuitry may mediate the formation of memories for the spatial position of behaviourally relevant cues. Place cells, which encode place fields, provide essential information for navigation. They are found in CA1 (REF. 102) and CA3 (REF. 103), but cells with similar functional properties have been found in the subiculum[104–106], the septal presubiculum[107] and the parasubiculum[108,109]. In the HF, the size of a place field is related to the septotemporal position of the place cells: place cells in the septal HF have the smallest place fields, at intermediate septotemporal levels place fields are twice as big[110] and in the temporal HF they become even larger[103,110,111]. Place field size can be interpreted as a measure of spatial scale, indicating that environments might be represented at different spatial resolutions along the septotemporal axis of the HF.

A large number of non-overlapping, unique spatial representations of the environment are stored in the rather limited network of the HF, which creates a storage problem. It has been argued that in order to solve this problem the HF might make use of a universal map, presumably located outside the HF[102,112,113], that can be applied across environments. Based on the strong reciprocal connectivity between the EC and the HF, the EC (in particular the MEA) was considered a likely candidate for the location of this map, as this area was shown to receive predominantly visuospatial information from the POR[16]. Indeed, a disruption of the monosynaptic information flow from MEA layer III to CA1 affected long-term spatial-memory performance[114] and impaired place cell firing in CA1 (REF. 115). However, initial recordings in the EC did not reveal cells with a striking spatially modulated firing pattern[116,117], probably because these recordings did not cover the most dorsolateral portion of the MEA. The dorsolateral MEA was predicted to contain such cells because it is reciprocally connected both to the septal hippocampus (see the 'Circuitry' section), in which place cells are

Auto-associative network
A network of neurons with axon collaterals that terminate on dendrites of the parent cell.

Place cells
Principal neurons in the hippocampus and parahippocampus that fire whenever an animal is in a specific location in an environment (corresponding to the cell's 'place field').

most conspicuous, and to visuospatial cortical domains — for example, the POR[17,18]. Subsequent recordings in the dorsolateral part of the MEA indeed revealed grid cells[118]. Like place cells, grid cells show a gradual increase in grid field size from the dorsolateral MEA towards the ventromedial MEA[119] and, because of the predominant topology of the perforant path, the grid cells with the smallest grid field scale in the dorsolateral MEA connect to the place cells in the septal HF with the smallest place field scale. Similarly, the grid cells with the largest grid field scale in the ventromedial MEA connect to the place cells in the temporal HF with the largest place field scale.

Head-direction cells are a third class of cells involved in navigation. Head-direction cells were first discovered in the septal presubiculum[109,120], but directionally tuned cells have also been observed in the EC[95], the anterior and lateral dorsal thalamic nuclei[121–123], the lateral mammillary nucleus[124], the retrosplenial cortex[125] and the striatum[126]. This indicates that the directional signal is probably computed in brain networks outside the HF. The head-direction information from the mammillary bodies is crucial for place and grid cell functioning[124], and head-direction information from the presubiculum is important, although not indispensable, for the functional characteristics of place fields in CA1 (REF. 127). As the septal presubiculum also projects to other HF subregions, we propose that the firing properties of neurons in the DG, CA3 and the subiculum might also be affected by presubiculum lesions.

What more can the details of the circuitry tell us about the space-related functional properties of the network? A first hypothesis is that information from the head-direction system may enter the HF through at least two different routes. One route projects from the presubiculum directly to the HF and a second route runs indirectly to the HF through the projections from layers II and III of the EC. In order to decide which of these routes provides the predominant directional input to the HF, the reported effects of presubiculum lesions on CA1 place cell firing[107] should be compared with the effect of presubiculum lesions on the spatial-firing properties of MEA neurons. If MEA neuron firing is not affected by such lesions, the direct route from the presubiculum to CA1 is more likely to be the predominant input pathway for directional information to the HF. However, if the firing properties of MEA neurons do change as a result of presubiculum lesions, the CA1 firing properties after a presubiculum lesion should be compared with the CA1 firing properties after a selective MEA lesion[115] and after a combined presubiculum and MEA lesion.

Another prediction based on the PHR–HF network characteristics is that the place-specific firing of CA1 should be stronger at its proximal end than at its distal end, as the MEA preferentially projects to the proximal portion of CA1 and place-specific firing in CA1 strongly depends on the direct input from the MEA[115,128,129]. By contrast, the preferential LEA-to-CA1 projection pattern predicts that non-spatial information about external stimuli is processed in the distal CA1. Preliminary data show that the firing of cells in the proximal (MEA-recipient) CA1 is indeed significantly more affected by spatial information than the firing of cells in the distal CA1 (REF. 101). A similar type of prediction can be made for the subiculum, as the LEA projects to the proximal part of the subiculum and the MEA projects to its distal part. On the basis of this topology, the most prominent place cells are expected to be found in the distal subiculum. One study found subtle differences in the spatial properties of cells in the proximal versus the distal subiculum[104]. There are several explanations for why the difference was only small, but the best explanation is probably the extensive but underexposed and not very well studied intrinsic recurrent network in the subiculum[130].

Temporal dynamics. Some of the underexposed PHR–HF connections are likely to be involved in the temporal synchronization of neuronal firing between brain areas. Synchronized firing is essential for the coordination of spatially distributed networks and is generally achieved through neuronal oscillations. By synchronizing excitatory periods across regions, oscillations may facilitate the transfer of information in the PHR–HF network[131]. Furthermore, oscillations promote coincident firing among cells, which is likely to be important for inducing synaptic plasticity (for example, see REF. 132) and memory consolidation[133]. One of the prerequisites for the occurrence of oscillations is the interaction between excitatory glutamatergic neurons and inhibitory GABA (γ-aminobutyric acid)-ergic interneurons[134]. Different classes of GABAergic neurons can be characterized in the hippocampus according to their distinct firing patterns during behaviourally relevant oscillations such as theta oscillations, gamma oscillations and sharp wave ripples[135–138]; projections from these interneurons to different targets synchronize the firing of large numbers of pyramidal cells[135]. Although most research on GABAergic cells is carried out on interneurons that project locally in one sub-area, recent evidence showed the existence of long-range GABAergic projection neurons that cross the sub-area border and are involved in the coordination of spike timing across sub-areas[139].

Although most tract-tracing studies do not reveal whether a projection is excitatory or inhibitory, an indication of the excitatory or inhibitory nature of a connection can be derived from the layers of origin and termination. For example, the CA1-to-CA3 backprojections discussed in the 'Circuitry' section arise not from the (excitatory) glutamatergic principal cell layer, but mainly from neurons located in the stratum oriens of CA1 (REFS 64,66,67,75), and project to the stratum radiatum and stratum oriens of CA3. An *in vivo* labelling study showed the same locus of origin and termination for CA1 GABAergic neurons projecting to CA3 (REFS 77,140–142). Also, in the stratum radiatum of CA1, cells project to the DG and the subiculum. GABAergic cells have been reported to reside in the CA1 stratum radiatum with axons that radiate to the molecular layer of the DG and the subiculum[143].

Because the layer of origin of these CA1 neurons seems to be a reliable predictor of GABAergic connections, it is likely that other projections that do not start

Grid cells
Neurons in the entorhinal cortex that fire strongly when an animal is at one of several specific locations in an environment and that are organized in a grid-like fashion.

Head-direction cells
Neurons that fire only when the animal's head points in a specific direction in an environment.

Mammillary bodies
A pair of nuclei in the hypothalamus, strongly connected to the HF and the anterior complex of the thalamus, that are involved in recognition memory.

Theta oscillations
Rhythmical changes at 5–12 Hz in network activity, as observed in the electroencephalogram, characteristic of the hippocampal network communicating with various cortical and subcortical networks in the brain.

Gamma oscillations
Rhythmical oscillations of 25–70 Hz observed in the electroencephalogram.

Ripple oscillations
Short-lasting bursts of field oscillations (~140–200 Hz) in the mammalian hippocampus and parahippocampus that occur during rest or slow-wave sleep.

in the principal cell layer are also GABAergic. This can be used to discover the existence of other inhibitory projections. In the interactive diagram (see Supplementary information S1 (figure)) one can observe that, in the hippocampus, cells in the hilus of the DG project to CA1. There are also reports of projections from the HF to the PHR that do not start in the principal cell layer of the HF: cells in the molecular layer of the subiculum project to the parasubiculum, the presubiculum and the POR. Moreover, cells in the stratum oriens and the stratum radiatum of CA1 and the molecular layer of the subiculum project to the LEA and the MEA. We suggest that these connections indeed originate from long-range GABAergic neurons, and are capable of functionally coupling the PHR–HF subregions and coordinate oscillations over the entire PHR–HF network.

Conclusions and future directions

Comprehensive knowledge of the organization of the PHR–HF connectivity is of pivotal importance for elucidating PHR–HF function. Such detailed knowledge of PHR–HF circuits will help us to understand how these circuits are engaged in spatial processing and temporal dynamics, as well as in other functions that have been associated with the region, such as episodic memory[144], crossmodal memory[145], recollection and recognition[146], memory for the temporal order of events[147–150] and visual perception of conjunctions[151]. Moreover, the PHR and HF are implicated in various disorders, such as Alzheimer's disease[152], epilepsy[153], schizophrenia[154] and depression[155]. Knowing the changes in connection patterns within and between these regions may help us to understand the underlying mechanisms of these PHR–HF-related disorders and consequently enhance the possibilities for treating them. This Review and the complementary knowledge base may facilitate the study of altered connectivity in animal models for diseases that involve the PHR and HF.

Although topological information is available in the interactive diagram for a large number of connections, increasing the knowledge base of PHR–HF connectivity is an important requirement for future functional understanding of these regions. Although currently all connections in the interactive diagram are displayed as if they are of equal density, we aim for future versions of the diagram (which will be available on our website) to differentiate between strong and weak connections. Unfortunately, connectional density is often not reported quantitatively in the anatomical literature, and even when it is reported it is a subjective observation that is difficult to compare between studies. Second, we aim to incorporate *in vivo* and *in vitro* electrophysiological data into future versions of this knowledge base so that it will contain information about the excitatory or inhibitory role of connections. Third, the current version of the diagram displays only the layers of origin and termination, but each region and layer consists of several cell types. We aim for future versions of the diagram to contain a description of pre- and postsynaptic cell types. Implementing these improvements requires extensive fundamental research into the cytoarchitectonic and connectional properties of the region, but this investment will have a tremendous impact on advancing our functional understanding.

An ever-increasing amount of anatomical knowledge brings with it several difficulties. One consequence of the overwhelming number of reported connections is that attention focuses on a selection of the connections whereas others fall into disuse, especially those for which the functional relevance is not entirely clear, such as some of the recurrent collaterals in the HF subregions. A knowledge base such as the one presented in this Review can help to prevent the loss of valuable knowledge and inspire creative minds to come up with new solutions for outstanding problems in the field.

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REVIEW
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Architecture of the Entorhinal Cortex A Review of Entorhinal Anatomy in Rodents with Some Comparative Notes

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The entorhinal cortex (EC) is the major input and output structure of the hippocampal formation, forming the nodal point in cortico-hippocampal circuits. Different division schemes including two or many more subdivisions have been proposed, but here we will argue that subdividing EC into two components, the lateral EC (LEC) and medial EC (MEC) might suffice to describe the functional architecture of EC. This subdivision then leads to an anatomical interpretation of the different phenotypes of LEC and MEC. First, we will briefly summarize the cytoarchitectonic differences and differences in hippocampal projection patterns on which the subdivision between LEC and MEC traditionally is based and provide a short comparative perspective. Second, we focus on main differences in cortical connectivity, leading to the conclusion that the apparent differences may well correlate with the functional differences. Cortical connectivity of MEC is features interactions with areas such as the presubiculum, parasubiculum, retrosplenial cortex (RSC) and postrhinal cortex, all areas that are considered to belong to the "spatial processing domain" of the cortex. In contrast, LEC is strongly connected with olfactory areas, insular, medial- and orbitofrontal areas and perirhinal cortex. These areas are likely more involved in processing of object information, attention and motivation. Third, we will compare the intrinsic networks involving principal- and inter-neurons in LEC and MEC. Together, these observations suggest that the different phenotypes of both EC subdivisions likely depend on the combination of intrinsic organization and specific sets of inputs. We further suggest a reappraisal of the notion of EC as a layered input-output structure for the hippocampal formation.

Keywords: parahippocampal region, hippocampus, connectivity, primate, rodent

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INTRODUCTION

The denomination "entorhinal cortex (EC)" (Brodman's area 28) is based on the fact that it is (partially) enclosed by the rhinal (olfactory) sulcus. Interest in the EC arose around the turn of the 20th century when Ramón y Cajal, described a peculiar part of the posterior temporal cortex that was strongly connected to the hippocampus by way of the temporo-ammonic tract (Ramón Y Cajal, 1902; Witter et al., in press). Cajal was struck by this massive connection

and he therefore suggested that the functional significance of the hippocampus had to be related to that of EC or the sphenoidal cortex/angular ganglion, as he called it at that time. Today, EC is conceived as the nodal point between the hippocampal formation on the one hand and a variety of cortical areas on the other hand. Multimodal, as well as highly processed unimodal sensory inputs converge at the level of neurons in the superficial layers of the EC. This input is conveyed by the neurons in layers II and III of EC to all subdivisions of the hippocampal formation (Insausti et al., 2004; van Strien et al., 2009; Cappaert et al., 2014; Strange et al., 2014). The hippocampal fields CA1 and subiculum are the main source of projections that return to layer V of EC, with a less dense projection to layers II and III. Layer V neurons in turn are the main origin of EC projections to widespread cortical and subcortical domains in the forebrain (Rosene and Van Hoesen, 1977; Kosel et al., 1982; Cappaert et al., 2014).

EC comprises different subdivisions, charaterized by connectivity with functionally different sets of cortical and subcortical areas in the brain. This has led to the now quite widely accepted concept of parallel input/output channels, mediated by way of perirhinal and postrhinal (rodents) or parahippocampal cortex (primates; Witter et al., 1989a, 2000; Naber et al., 1997; Eichenbaum et al., 2012; Ranganath and Ritchey, 2012). Recent electrophysiological recordings in the lateral and medial EC (LEC and MEC respectively; see below for definitions) of rodents show that cells in MEC are predominantly spatially modulated. In contrast, in LEC such modulation is essentially absent, with neuron-firing correlating to objects in context (Fyhn et al., 2004; Deshmukh and Knierim, 2011; Knierim et al., 2013; Tsao et al., 2013; Moser et al., 2014). Does this phenotypical difference between the two EC components reflect input differences, or differences in local circuits and cell types, or could this phenotypical separation be the result of interactions between these two parameters. In this review, we aim to address specifically this question by providing a comprehensive description of EC, its intrinsic organization in relation to input and output organizations. We mainly focus on data from studies in rodents, although occasional comparative remarks are inserted when considered relevant for the narrative of the article.

DEFINITION OF THE ENTORHINAL CORTEX, SUBDIVISIONS AND OVERALL ARCHITECTURE

There are different ways to define a cortical area, using different criteria, such as location, connectivity, cyto- and chemoarchitecture. Applying all of these approaches has resulted in a variety of borders, subdivisions and description of layers. Architectural parcellation schemes are useful tools to relate experimental data to standard locations in the brain (Bjaalie, 2002; van Strien et al., 2009; Zilles and Amunts, 2010; Kjonigsen et al., 2011, 2015; Boccara et al., 2015). Connection-based subdivision schemes may relate closer to our understanding of functional differences between areas (see below). In view of the strong implications of the human EC in a variety of brain diseases (Braak and Braak, 1992), the development of adequate animal models for such diseases depends strongly on our capabilities to extrapolate the definition of the EC from rodents to non-human and human primates. Therefore, combinations of the different approaches mentioned above will likely provide the most reliable concept for subdividing EC.

An apparently good lead, since it has withstood over a century of arguments, is the definition of EC based on hippocampal connectivity, as originally suggested by Ramón Y Cajal (1902, 1911). In view of increasing insights into the connectivity of the hippocampal formation and its subdivisions, we follow the well-established practice in rodents to take the differential distribution of EC projections to the dentate gyrus as a good defining criterion for two main subdivisions of EC. These are nowadays referred to as LEC and MEC (Steward, 1976; Witter, 2007). Unfortunately, in the monkey, the terminal distribution of the entorhinal-to-dentate projection does not provide such a clear criterion to functionally subdivide EC (Witter et al., 1989b). Potentially in line with this, cytoarchitectural division schemes tend to differentiate more than two subdivisions (Amaral et al., 1987; Rosene and Van Hoesen, 1987). However, the second entorhinal-hippocampal projection, connecting the two entorhinal domains to area CA1 and the subiculum in all mammalian species studied, including primates, shows a strikingly preserved topology along the transverse axis of both hippocampal fields. Projections emerging from a posteromedial location in EC target the proximal CA1, i.e., close to DG, and distal subiculum, whereas an anterolateral origin in EC maps onto the distal CA1 and adjacent proximal subiculum (human: Witter et al., 2000; Maass et al., 2015; monkey: Witter and Amaral, 1991; rat: Naber et al., 2001; van Strien et al., 2009).

Other connectivity patterns have been proposed to functionally subdivide EC as well, one being the input from the presubiculum. In all non-primate mammalian species studied so far, including rat, guinea pig and cat, the innervation of EC by presubicular fibers is restricted to a more caudal and dorsal portion that coincides with a cyto- and chemoarchitectonically well defined area, now called MEC (Shipley, 1975; Köhler, 1984; Room and Groenewegen, 1986). Also in the monkey, inputs from the presubiculum distribute to only a restricted posterior portion of EC (Amaral et al., 1984; Saunders and Rosene, 1988; Witter and Amaral, unpublished observations), and this area may thus represent the homolog of MEC as defined in non-primates. Recent connectional MRI studies in humans have pointed to a comparable connectional bipartite system separating anterolateral from posteromedial EC, showing clear differences with respect to connectivity measures with perirhinal and parahippocampal cortex, resembling those reported in rodents (Naber et al., 1997; Maass et al., 2015; Navarro Schröder et al., 2015).

Cytoarchitectural data reveal that in all species studied, two entorhinal areas can be differentiated and that these share cytoarchitectonic features with the two entorhinal areas

defined by Brodmann as areas 28a and b (Brodmann, 1909). One can easily recognize a posteromedial area characterized by a very regular six-layered structure and a homogenous distribution of neurons in all layers, typical for area 28b or MEC. Layer II of MEC comprises a mixture of excitatory medium-sized pyramidal neurons and large multipolar neurons that have become known as stellate cells (SCs). On the opposite, anterolateral side, the laminar structure is comparable, but much less regular, resembling the cytoarchitecture of area 28a or LEC. In the latter portion, layer II comprises a mixture of large multipolar neurons, nowadays in rodents referred to as fan cells, pyramidal and medium-sized multipolar neurons. At some locations, these cell types seem to cluster into sublayers (referred to as IIa and IIb, or II and IIIa; Kobro-Flatmoen and Witter, 2017). Depending on the species, one or several additional subdivisions have been described, similar to what was mentioned above for the monkey (Lorente de Nó, 1933; Insausti et al., 1997). Note that the terms LEC and MEC do not simply reflect a particular position in anatomical or stereotaxic space. In many species, the two areas, defined by their combined architectural and hodological features occupy a more rostrolateral (LEC) vs. a more caudomedial position (MEC).

CONNECTIVITY OF THE TWO ENTORHINAL SUBDIVISIONS

Both LEC and MEC project to the hippocampus, and the axons form synapses on neurons in all hippocampal subfields. Neurons in layer II are the main source of the entorhinal projections to the dentate gyrus and fields CA2 and CA3, and neurons in layer III give rise to the entorhinal projections to CA1 and subiculum (note that a small number of neurons in deeper entorhinal layers contribute to both projections). In view of a confusing nomenclature that has developed over the years to describe these different projection systems (for a recent description and discussion, see Witter et al., in press), in the present article, we differentiate between the EC-layer II projection and the EC-layer III projection. Regarding the EC-layer II projection, we know that single layer II cells project to both the dentate gyrus and CA2/CA3 (Tamamaki and Nojyo, 1993). Whether such a collateral organization is true for the layer III projection to CA1 and subiculum is unclear. In view of this striking layer-separation in the origin of the EC to hippocampus projections, we feel that a description of intrinsic and extrinsic connectivity of LEC and MEC might benefit from a layered approach. In the following, we focus on the main cell layers II, III and V (for a description of layers I and VI, the reader is referred to Canto et al., 2008; Cappaert et al., 2014).

Extrinsic Connections

The two entorhinal divisions differ with respect to their major extrinsic cortical and subcortical connections (for recent detailed overviews in the rat, see Kerr et al., 2007; Cappaert et al., 2014; for broader comparative overviews of cortical connectivity in a functional context, see Eichenbaum et al., 2012; Ranganath and Ritchey, 2012). Here we focus on a description of the distribution of main cortical inputs and their laminar preference of termination. Superficial layers of EC receive a substantial input from olfactory structures including the olfactory bulb, the anterior olfactory nucleus, and the piriform cortex (Haberly and Price, 1978; Kosel et al., 1981). Olfactory axons preferentially terminate laterally and centrally in LEC and in MEC, avoiding the most caudodorsal portion of MEC (Kerr et al., 2007). Olfactory fibers mainly distribute to layer I, where they make synaptic contacts with dendrites of neurons in layers II and III (Wouterlood and Nederlof, 1983). Other superficially terminating inputs to dorsolateral parts of LEC originate from insular cortex (Mathiasen et al., 2015), perirhinal cortex (Naber et al., 1999; Pinto et al., 2006) and orbitofrontal cortex (Hoover and Vertes, 2007, 2011; Kondo and Witter, 2014). Interestingly, the orbitofrontal and insular projections to LEC mainly terminate anteriorly, and close to the rhinal fissure. Parietal cortex projects moderately to LEC and MEC, terminating close to the rhinal fissure, preferentially in layers I and V (Olsen et al., 2017). Superficial layers of MEC receive inputs from the orbitofrontal cortex, but only from the ventral part (Kondo and Witter, 2014), postrhinal cortex (Koganezawa et al., 2015) and pre- and parasubiculum (Caballero-Bleda and Witter, 1993). The latter two inputs not only terminate on dendrites of neurons in layers II and III, but also influence neurons in layer V (Canto et al., 2012), and such a connectional scheme might hold true for all superficially terminating inputs. This however remains to be established, but the possibility points to a potentially relevant role for layer V neurons as integrators of entorhinal inputs, since they also are the recipients of other major cortical inputs distributing to layer V. These include inputs from infralimbic and prelimbic cortex, apparently innervating LEC and MEC almost equally dense. LEC layer V receives a denser input from anterior cingulate cortex, whereas the retrosplenial innervation almost exclusively distributes to MEC layer V (Wyss and Van Groen, 1992; Vertes, 2004; Jones and Witter, 2007), which also receives a weak to moderate input from visual cortex (Kerr et al., 2007; Olsen et al., 2017).

Intrinsic Networks Layer II

Principal cells in both subdivisions of EC come in two chemical types, calbindin- and reelin-expressing cells. In MEC, calbindin-positive cells and reelin-positive cells appear to be grouped in patches, and in LEC the two cell types are more or less confined to two separate sublayers, reelin cells in layer IIa and calbindin cells in layer IIb. The reported clustering of calbindin-positive neurons is particularly striking in limited parts of MEC and is more striking in mice than in rats or other species. Only in mouse MEC the calbindin-positive neurons are located superficial to the reelin positive neurons (**Figure 1A**; Tunon et al., 1992; Fujimaru and Kosaka, 1996; Wouterlood, 2002; Ramos-Moreno et al., 2006; Kitamura et al., 2014; Ray et al., 2014; Leitner et al., 2016). EC in humans is known for its wart-like bumps or verrucae (Retzius, 1896; Klinger, 1948; Solodkin and Vanhoesen, 1996; Naumann et al., 2016), which in the largest part of EC, located centrally along

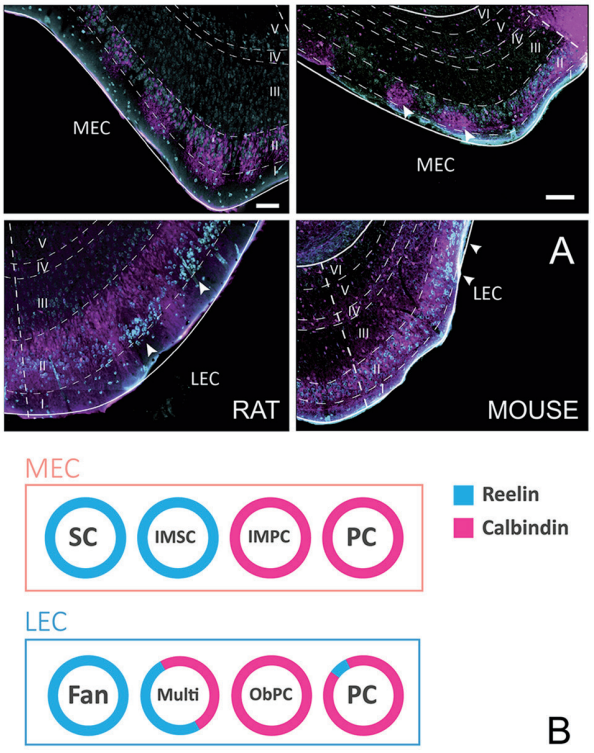

FIGURE 1 | Layer II cells come in two, chemically defined types, reelin- and calbindin-positive. **(A)** Coronal sections taken through entorhinal cortex (EC) of the rat (left) and mouse (right), stained for reelin (cyan) and calbindin (magenta). Note the different position of the two cell populations in the two species and the two subdivisions of EC. In the rat medial EC (MEC; top-left), the two populations are intermingled with a tendency for both types to cluster somewhat. In contrast, in mouse MEC (top-right), calbindin-positive cells form clear clusters (white arrowheads) that are located superficial to the reelin-positive neurons. In lateral EC (LEC) of the rat (bottom-left), reelin-positive cells form superficially positioned clusters (white arrowheads), separated by calbindin-positive dendritic bundles belonging to the deeper positioned, equally dispersed calbindin-positive neurons. In LEC of the mouse (bottom-right), a more equal distribution is seen, although two superficially located reelin clusters are present (white arrowheads). Scale bars equal 100 μm. **(B)** Schematic representation of the relationships of morphologically, electrophysiologically and connectionally defined cell types, and their chemical phenotype in LEC and MEC. Abbreviations: Fan, fan cell; IMSC, intermediate stellate cell; IMPC, intermediate pyramidal cell; multi, multipolar cell; ObPC, oblique pyramidal cell; PC, pyramidal cell; SC, stellate cell.

the anteroposterior and lateromedial axes, are composed of the large multipolar reelin positive layer II cells, described as the pre-alfa neurons by Braak (Braak and Braak, 1985; Tunon et al., 1992; Kobro-Flatmoen et al., 2016; Naumann et al., 2016). Moreover, the marked clustering of calbindin-positive neurons in all species studied is limited to a restricted posterior part of MEC (Naumann et al., 2016). In our view, it is therefore confusing to refer to calbindin-positive cells in layer II as island cells embedded in an ocean of reelin-positive cells (Kitamura et al., 2014), since this organization is likely opposite for the larger part of EC. Reelin-positive cells in both entorhinal areas project to the dentate gyrus and CA3, whereas calbindin-positive neurons project to several other targets including the CA1 and the contralateral EC, the olfactory bulb and piriform cortex (Varga et al., 2010; Kitamura et al., 2014; Fuchs et al., 2016; Leitner et al., 2016; Ohara et al., 2016). The two chemically defined cell groups are composed of several morphological subgroups that can be distinguished based on somatic and dendritic features (Canto and Witter, 2012a,b; Fuchs et al., 2016; Leitner et al., 2016).

In MEC, SCs make up the largest subgroup of principal cells. They have multiple primary dendrites that radiate out from a round soma. SCs are typically reelin-positive and calbindin-negative. Medium to large pyramidal cells (PCs) make up the other main principal cell type in layer II of the MEC. PCs are typically calbindin-positive, although a few reelin-positive PC have been described (Fuchs et al., 2016; **Figure 1B**). There are at least two intermediate cell groups in between stellate and pyramidal morphologies, here referred to as intermediate SCs (IMSCs) and intermediate PCs (IMPCs). IMSCs all express reelin, but a few of them co-express calbindin, the IMPCs tend to be calbindin-positive, but are more diverse and come in both reelin-positive and reelin and calbindin co-expressing varieties. The four principal cell types in the MEC can also be distinguished from each other based on their electrophysiological profiles (Canto and Witter, 2012b; Fuchs et al., 2016).

In LEC layer II, there are also at least four subgroups of principal cells (Canto and Witter, 2012a; Leitner et al., 2016). Fan cells are similar in morphology to SCs, but lack a distinctive basal dendritic tree (Tahvildari and Alonso, 2005; Canto and Witter, 2012a). Most are reelin-positive, though some may express calbindin. PCs make up the other large group of principal cells in LEC, they are morphologically similar to those described in MEC. They are largely calbindin-positive, but some may be reelin-positive. Oblique PCs (ObPCs) and multipolar cell make up the intermediate cell types in the LEC (Canto and Witter, 2012a; Leitner et al., 2016). Oblique pyramidals display a morphology similar to PCs, but are tilted relative to the pial surface, and they predominantly express calbindin. Multipolar cells, on the other hand, have a more diverse morphology, and express both calbindin and reelin (**Figure 1B**). Electrophysiologically, the four cell groups in LEC are not as easily distinguishable as in MEC, however recent data suggest that there may be subtle physiological differences between the overarching reelin and calbindin classes (Tahvildari and Alonso, 2005; Canto and Witter, 2012a; Leitner et al., 2016).

Similar to what has been reported for neocortical areas, EC has been suggested to contain three main subgroups of interneurons, parvalbumin (PV), somatostatin (SOM) and 5HT3a expressing cells (Rudy et al., 2011; Fuchs et al., 2016; Leitner et al., 2016). PV-positive interneurons constitute approximately half of the interneuron population across EC, making them the largest subgroup of interneurons in the area (Wouterlood et al., 1995; Miettinen et al., 1996). Layer II of MEC has a large number of PV expressing somata and heavy neuropil staining. Layer II of LEC has comparatively weak PV staining, with

few somata and light neuropil staining. Particularly layer IIa appears to lack PV-positive cells (Wouterlood et al., 1995; Fujimaru and Kosaka, 1996; Miettinen et al., 1996; Leitner et al., 2016). In both LEC and MEC, there is a clear gradient of PV staining, with portions close to the rhinal fissure expressing more than ventral portions (Wouterlood et al., 1995; Fujimaru and Kosaka, 1996; Leitner et al., 2016). A comparable, and strikingly strong gradient has been reported in relation to the collateral and rhinal sulcus in primates (human: Tunon et al., 1992; monkey: Pitkanen and Amaral, 1993; for a detailed comparative description, see Kobro-Flatmoen and Witter, 2017).

Like PV cells in other parts of the brain (Hu et al., 2014), those in layer II of MEC are known to display a fast spiking physiological profile (Couey et al., 2013; Pastoll et al., 2013; Armstrong et al., 2016; Fuchs et al., 2016; Leitner et al., 2016). The existence of PV-positive baskets surrounding principal cells in layer II is supported by both histological and electrophysiological studies (Jones and Bühl, 1993; Wouterlood et al., 1995; Varga et al., 2010; Armstrong et al., 2016; Fuchs et al., 2016). Another type of basket cell in layer II of MEC is the CCK-expressing basket cell (Varga et al., 2010; Armstrong et al., 2016). These cells are less abundant than PV-expressing cells, and constitute a subgroup of the 5HT3aR expressing interneurons (Lee et al., 2010). Whereas CCk-positive basket cells preferentially target calbindin-positive principal cells, single PV-positive basket cells innervate both reelin- and calbindin-positive neurons (Armstrong et al., 2016). Basket cells have also been described in layer II of the LEC, but no details are available about different types and abundance, nor how they are part of the LEC microcircuit.

A second, common type of GABAergic interneuron that expresses PV in layer II, also present in layer III, is the chandelier or axo-axonic cell. Chandelier cells are characterized by vertical aggregations of axonal boutons, called candles which mainly make synapses on the initial axon segments of principal cells. In MEC, both vertical and horizontal chandelier cells are present, and in LEC the horizontal subtype is dominant. The local axon branches of these neurons are largely confined to layers II and III (Soriano et al., 1993).

Immunohistochemical studies describing the distribution of somatostatin expressing somata in EC are conflicting, particularly with regards to distribution in superficial layers. However, no major differences between entorhinal subdivisions have been described (Köhler and Chan-Palay, 1983; Wouterlood and Pothuizen, 2000). Somatostatin cells in MEC are generally multipolar low threshold spiking neurons (Couey et al., 2013; Fuchs et al., 2016). Available data indicate that only a small percentage of somatostatin neurons in EC are GABAergic (Wouterlood and Pothuizen, 2000), but our own data in mice show that most somatostatin neurons in EC are GABAergic (**Figure 2**). The last major interneuron group in EC, the 5HT3aR cells, consist of several subgroups, including calretinin-, VIP- and CCK-expressing cells (Lee et al., 2010; Fuchs et al., 2016; Leitner et al., 2016). 5HT3aR cells in layer II of MEC have diverse morphological and physiological profiles (Canto et al., 2008; Fuchs et al., 2016).

The regular grid pattern, typically seen in layer II of MEC has been hypothesized to emerge from the structure of microcircuits within layer II (Fuhs and Touretzky, 2006; McNaughton et al., 2006; Burak and Fiete, 2009; Bonnevie et al., 2013;

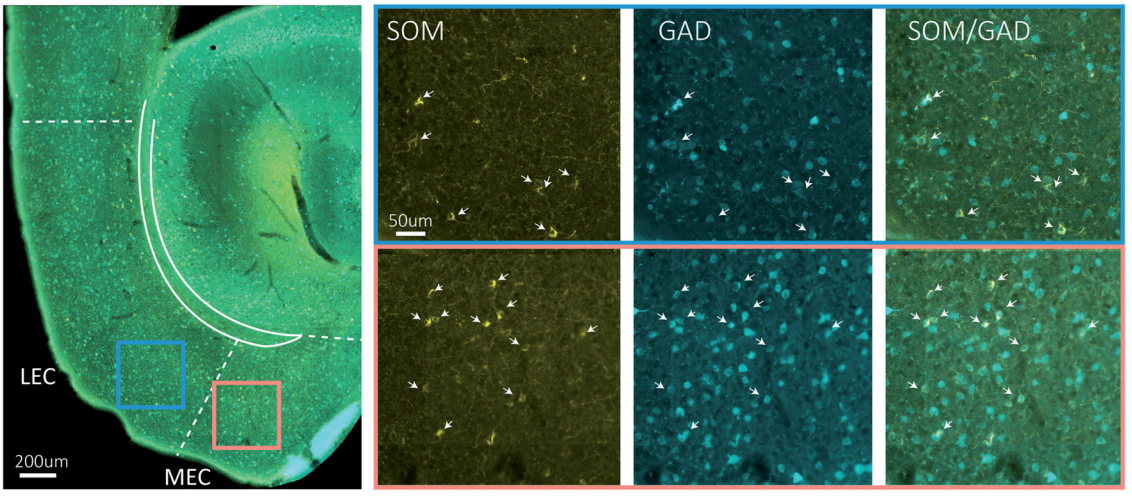

FIGURE 2 | Somatostatin neurons are GAD67 positive. The left hand side main panel shows a low power image of a horizontal section obtained from a GAD67 transgenic line expressing GFP (Tanaka et al., 2003), stained for the expression of somatostatin. The colored squares indicate the position of the high power images shown on the right. Blue square is LEC, red square is MEC. The solid blueish staining at the edge of EC is an artifact due to overlying cerebellar tissue. On the right hand side, high power images show the indicated areas in LEC and MEC in three different fluorescent channels from left to right: somatostatin (yellow), GFP (cyan) and overlay of somatostatin and GFP. Scale bars equal 200 μm in the left main panel and 50 μm for the six panels on the right-hand side.

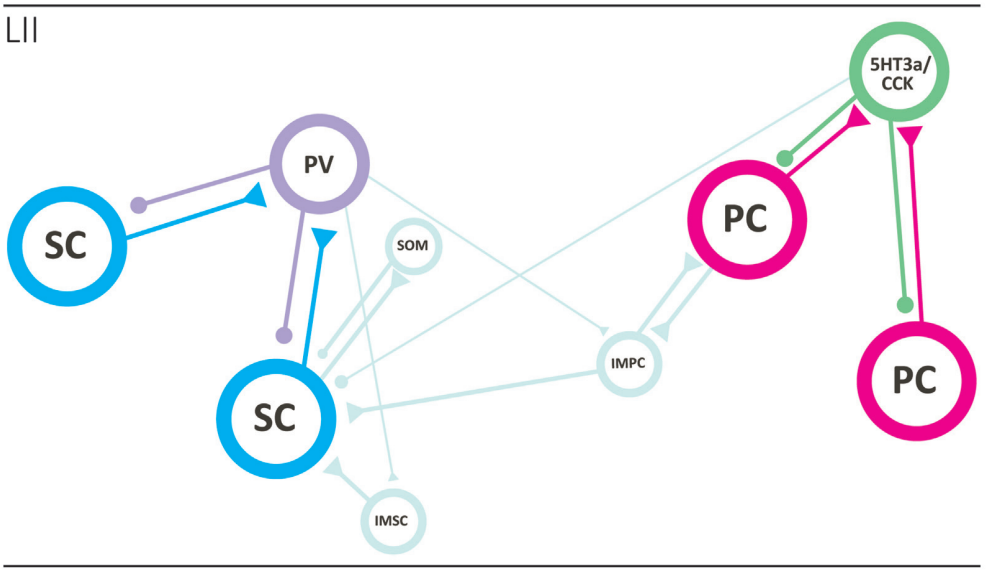

FIGURE 3 | Local circuits in layer II of MEC. The two main principal neurons, stellate (cyan; left side) and pyramidal (magenta; right side) cells form two specific networks. SCs are not monosynaptically connected and that seems true for PCs as well. They are disynaptically connected preferentially by way of different interneuron subtypes, the fast-spiking PV positive basket cell in case of SCs and the 5HT3a/CCK-type in case of pyramidal neurons. The two networks are likely interconnected by way of intermediate pyramidal neurons (light blue), and PV interneurons may also target intermediate pyramidal and SCs. See text for further details. Abbreviations: IMSC, intermediate stellate cell; IMPC, intermediate pyramidal cell; PC, pyramidal cell; PV, parvalbumin expressing fast spiking basket cell; SC, stellate cell; SOM, somatostatin-expressing interneuron; 5HT3a/CCK, basket cell that expresses CCK and likely belongs to larger group of interneurons that express the 5HT3a receptor.

Couey et al., 2013). The majority of grid cells in MEC are observed in layer II (Hafting et al., 2005; Sargolini et al., 2006), and the anatomical correlates of grid cells likely comprise both stellate-like and pyramidal-like cells (Domnisoru et al., 2013; Schmidt-Hieber and Häusser, 2013; Tang et al., 2014). The local circuit of SCs has been probed in several studies using *in vitro* patch clamp recordings, and it is now well established that individual SCs do not form monosynaptic connections with other SCs. Communication between SCs occurs through an intermediate inhibitory interneuron, in a mechanism by which activation of one or more SCs evokes disynaptic inhibitory currents in neighboring SCs. Paired recordings have revealed strong connectivity in both directions between SCs and fast-spiking cells and, to a much lesser extent, between SCs and low-threshold spiking interneurons (Couey et al., 2013; Pastoll et al., 2013; Fuchs et al., 2016). The functional disynaptic link that illustrates the core principle of the stellate microcircuit is mediated by a single type of inhibitory neuron, the PV positive fast spiking cell (**Figure 3**; Buetfering et al., 2014; Armstrong et al., 2016).

The local network of PCs has been explored using similar methods, and like the SC network, very sparse monosynaptic connectivity was detected between PCs. These results suggest that the general principle of disynaptic connectivity as described for the SC network also applies to the layer II PCs. An important distinction however is that PCs seem to communicate through different subsets of interneurons. In contrast to SCs, PCs are not connected, in either direction, to PV positive fast-spiking cells or somatostatin positive low threshold spiking cells, but instead form synaptic connections solely with the heterogeneous $5HT_{3A}$ expressing population of interneurons (**Figure 3**; Fuchs et al., 2016).

Synaptic interaction between the pyramidal and SC networks is limited, as available data points to little monosynaptic connectivity between stellate and PCs (Couey et al., 2013; Fuchs et al., 2016). This suggest the existence of two isolated subcircuits within layer II of MEC, where information relayed to the dentate gyrus by reelin positive SCs is processed separately from information relayed by calbindin positive PCs to other downstream areas. However, it should be kept in mind that the networks may be coordinated through one of the intermediate cell types, e.g., the IMPCs, which have been shown to form synaptic connections with both pyramidal and SCs (**Figure 3**; Fuchs et al., 2016).

If the local microcircuit design of layer II MEC excitatory cells is crucial for generating grid cell firing, the absence of grid cells in LEC predicts a different organization of the layer II principal cell microcircuit. Given the observation that inhibition dominates microcircuits of both pyramidal and SCs in MEC, albeit provided by different types of interneurons, comparable cell types in the LEC, e.g., the fan and PC, may have a circuit structure where monosynaptic connectivity prevails. Our preliminary data from paired recordings of fan cells indicates that direct communication between cells of this type is present, but not prevalent (Nilssen et al., 2015). Potential microcircuit

differences between layer II of MEC and LEC might also reflect different contributions from the local interneuron population. In LEC, 5HT3aR expressing interneurons constitute the largest interneuron group in layer II, unlike in the MEC, where PV cells are thought to be the predominant interneuron group (Leitner et al., 2016). This finding indicates that the inhibitory systems in MEC and LEC layer II are dominated by different subtypes of interneurons.

Layer III

Compared with what is known about neurons and connectivity in layers II and V, Layer III is still largely terra incognita. Layer III in both LEC and MEC comprises a homogenous population of spiny excitatory pyramidal neurons that project to CA1 and subiculum (Tahvildari and Alonso, 2005; Canto and Witter, 2012a,b; Tang et al., 2015). Layer III neurons also project contralaterally to the hippocampus and EC (Steward and Scoville, 1976). About 40% of the layer III hippocampal projecting cells in MEC send collaterals to the contralateral MEC (Tang et al., 2015). The axons of the commissural projecting cells in MEC apparently distribute mainly to layer III, thus contrasting to the small percentage of commissural calbindin-positive neurons in layer II, of which the axons preferentially distribute in layer I of the contralateral MEC (Fuchs et al., 2016). In addition, layer III also contains a population of non-spiny PCs, sending axons towards the angular bundle. Collaterals originate from the main axon close to the cell body and those traveling towards the superficial layers distribute over the own dendritic extent (Gloveli et al., 1997). The third principal neuron type in layer III is formed by multipolar neurons. These contribute to the hippocampal projections (Germroth et al., 1989). Layer III contains a variety of interneurons, exhibiting various morphologies, including multipolar, pyramidal and bipolar neurons. Chemical characterization of layer III interneurons in the MEC shows that they express several markers including somatastatin, calbindin, vasoactive intestinal peptide and substance-P (Köhler and Chan-Palay, 1983; Köhler et al., 1985; Gloveli et al., 1997; Wouterlood and Pothuizen, 2000; Wouterlood et al., 2000; Kumar and Buckmaster, 2006).

The microcircuits of layer III are only sparsely known, but seem to be markedly different from those seen in layer II, showing a much stronger monosynaptic principal to principal neuron connectivity (van der Linden and Lopes da Silva, 1998; Dhillon and Jones, 2000; Kloosterman et al., 2003; Tang et al., 2015). Neurons in layer III are the main recipients of the local deep-to-superficial projections, which apparently predominantly originate from neurons in layer Vb (see below; Kloosterman et al., 2003; van Haeften et al., 2003). Currently, no correlations have been reported between morphology, connectional profile and electrophysiological *in vitro* and *in vivo* properties (Canto and Witter, 2012a,b; Tang et al., 2015).

Layer V

As described above, layer V is commonly subdivided into a layer Va and Vb. The superficial layer Va, adjacent to layer IV (lamina dissecans), comprises mainly large pyramidal neurons that are unequally distributed along the extent of both MEC and LEC. Cells in layer Vb appear smaller, more uniform in soma size and are more densely packed than their counterparts in layer Va (Canto and Witter, 2012a,b; Boccara et al., 2015).

In mice, the expression pattern of the transcription factors Etv1 and Ctip2 provide for the differentiation between two molecularly distinct sublayers Va and Vb, respectively. This organization prevails across the whole mediolateral and dorsoventral extent of EC (Ramsden et al., 2015; Surmeli et al., 2015; Onodera et al., 2016). In both MEC and LEC, layer Va cells are the major output neurons projecting to diverse cortical and subcortical structures. Surprisingly, layer Vb cells are selectively targeted by the outputs from the hippocampus, originating in CA1 and subiculum as well as by projections originating in layer II of EC (**Figure 4**; Surmeli et al., 2015; Onodera et al., 2016). In MEC, these layer II inputs apparently arise specifically from reelin positive MEC II SCs and not from the calbindin positive MEC II PCs (Surmeli et al., 2015). The latter report of axon collaterals from layer II SCs in layer V in mice conflicts with previous reports in rats and monkeys, that layer II SCs issue a well-developed axonal plexus in layers I and II, but that collaterals in layer V are sparse (Tamamaki and Nojyo, 1993; Klink and Alonso, 1997; Buckmaster et al., 2004; Canto and Witter, 2012b). Whether this points to species differences or a lack of sensitivity in the older studies is not known. Irrespective of the details of this circuit, MEC layer Vb neurons could be ideally suited to integrate inputs from superficial MEC and hippocampus. Own preliminary data show these network features to be true in LEC as well, and show that layer Vb neurons in both LEC and MEC innervate layer Va as well as layers II and III (Onodera et al., 2016), which is in line with sparse data indicating that neurons in layer Vb issue superficially directed axon collaterals (Hamam et al., 2000, 2002; Canto and Witter, 2012a,b). This indicates that at least a subpopulation of layer Vb neurons form a major component of the intrinsic deep to superficial circuit.

Layer V is also innervated by additional cortical projections from frontal and cingular domains (see above). Whereas information about the postsynaptic targets of these cortical inputs is sparse, projections from the retrosplenial cortex (RSC) to MEC layer V target, among others, spiny pyramidal neurons that issue axons to superficial layers (Czajkowski et al., 2013). If the assertion is correct that in particular layer Vb neurons are the main elements mediating this deep to superficial connection, it is logical to conclude that retrosplenial inputs terminate onto a subpopulation of Vb neurons (**Figure 4**). These data are thus in line with own preliminary observations that neurons in layer V receive convergent inputs from subiculum and RSC (Simonsen et al., 2012).

Layer Vb of both MEC and LEC also contains multipolar neurons (Hamam et al., 2000; Canto and Witter, 2012b) and a population of GABA-negative/calretinin positive neurons (Miettinen et al., 1997) providing additional markers for principal cell types in the layer V network. Electrophysiologically, PCs in layer V show regular spiking, strongly adapting

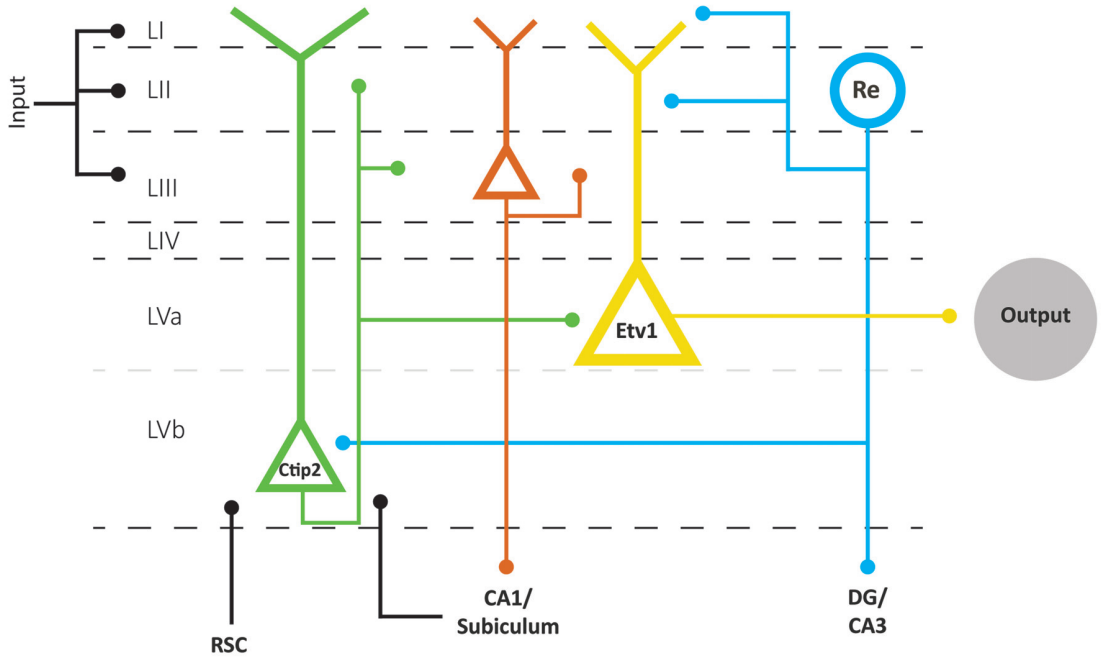

FIGURE 4 | Schematic representation of the layer V network as part of the EC network. Layer V comprises two sublayers Va and Vb, based on the differential expression of two transcription factors, Etv1 and Ctip 2, respectively. Layer Va neurons are the main origin of projections to cortical and subcortical structures in the brain. Layer Vb neurons receive inputs from the hippocampus and RSC and project locally to Va and superficial layers. Superficial inputs likely form synapses onto dendrites of principal neurons in layers II, III and V of EC. Neurons in layer II and III provide the main input to the hippocampus, which is returned to layer VB and subsequently made available to layer Va neurons, which originate the main outbound projections of EC. Neurons in layer Vb are also the main source of back projections to layer II and III neurons. The scheme clearly shows that we lack detailed connectional data on layer III as well as on input specificity to layer Va and Vb neurons. Abbreviations: Re, reelin-expressing neurons; RSC, retrosplenial cortex.

physiological profiles, whereas multipolar neurons respond to a depolarization with delayed firing and slow little adaptation (Egorov et al., 2002b). It is currently not known if any of these layer V cell types correlate with the electrophysiologically defined persistent firing neurons, which can be found in EC when muscarinic acetylcholine receptors are activated (Egorov et al., 2002a). Finally, we currently lack a detailed comparison of the organization of layer V in LEC and MEC. For example, what would be the functional implication that MEC layer Va hosts pyramidal neurons with extensive basal dendritic trees restricted to the somatic layer, whereas such a neuron type has not been reported in LEC (Hamam et al., 2000, 2002; Canto and Witter, 2012a,b; Surmeli et al., 2015).

CONCLUDING REMARKS

The comparison of main trends in extrinsic and intrinsic connectivity patterns of MEC and LEC suggests that the different phenotypes of both EC subdivisions likely depend on the combinatorial effects of small differences in intrinsic organization and substantial differences in extrinsic inputs. Although this conclusion and the following details are mainly based on studies in rodents, the more sparse data in non-human and human primates seem to support a comparable organization. To understand the functional relevance of the subtle intrinsic differences, more data are needed, for which we likely will depend on the emergence of even more specific genetic tools to identify and manipulate the activity of single classes of neurons. Eventually, detailed imaging studies in humans are expected to contribute to an increased understanding of the functional diversification within EC. The extrinsic input differences as summarized above are still in overall support with the notion that two functionally different input streams to the hippocampus are mediated by two entorhinal domains. MEC provides connectional routes with extensive posterior parts of the cortex, including posterior parahippocampal, retrosplenial, parietal and occipital networks, allowing the representation of intrinsically generated signals about perceived and/or planned movements in stable contexts. In contrast, LEC mediates routes to and from the hippocampus with more anterior parahippocampal, sensory and pre- and orbitofrontal domains, providing access to evaluated information about the ever-changing external world. From a functional anatomical perspective, the above provides a suitable framework to keep adding the details needed to mechanistically understand the role(s) of EC. The connectional scheme as presented here (**Figure 4**) assumes that the functionally different parts of EC share the network structure to mediate cortical-hippocampal interactions in a comparable matter. Neurons in layers II and III provide various combinations of information

to the hippocampal circuit, and a copy of that input is made available to neurons in layer V. The latter step might either be monosynaptic through inputs targeting the extensive apical tufts of some of the layer V pyramidal neurons or disynaptic through intrinsic projections from layer II (and layer III) to layer Vb. In view of the strict topology of the reciprocal connectivity between EC and CA1/subiculum, it is likely that at least some of these layer Vb neurons receive a hippocampally processed copy of that original input information. Layer Vb neurons are in a position to integrate those inputs with additional sets of information, and to send the resulting representations back to layers II and III. In case of layer Va neurons, which apparently are the origin of the main output pathway of EC, the hippocampally processed copy might be disynaptical, mediated through Vb neurons, and it is currently not known whether other inputs integrate at the level of these Va neurons. In view of their apical dendrites reaching the superficial layers of EC, it is likely that they, like layer Vb neurons, do receive superficially terminating inputs.

If correct, the connectional data strongly argue that differences in cortical inputs form a main feature underlying the phenotypic differences between LEC and MEC. However, we have not yet included the potential differences between LEC and MEC in local inhibitory architecture, as suggested by the yet sparse data on layer II. One additional feature of the proposed scheme needs to be discussed. The overarching strict reciprocal topology of the entorhinal-CA1-subicular network predicts that inbound information will be reciprocated with outbound information. It is exactly this last prediction, which is not supported by data. Admittedly, the available data are sparse, but the data obtained in the few studies in which this input-output dogma was addressed point to another direction. In one study in the cat, EEG recordings in freely behaving animals indicated a functional separation between LEC and MEC, where LEC is coupled to the olfactory domain, whereas MEC is coupled to the hippocampus (Boeijinga and Lopes da Silva, 1988). In more elaborate studies using the isolated guinea pig *ex vivo* brain preparation, olfactory stimulation resulted in a sequential activation in LEC, hippocampus and MEC, followed by LEC (Biella and de Curtis, 2000). These sparse data seem to indicate that hippocampal output, resulting from olfactory input, is preferentially distributed back to MEC, not to LEC. To our knowledge, this output pathway specificity has not been explored and thus presents us with a, yet underexplored, challenge, which might very well be open to imaging studies in the human.

AUTHOR CONTRIBUTIONS

All authors contributed to the discussions that formed the foundation of the manuscript and contributed to the writing of the manuscript and to figures. All figures with exception of 1A were made by BJ. MPW supervised the process and wrote the final version of the manuscript. All authors approved this final version.

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Comparative Contemplations on the Hippocampus

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Abstract
The hippocampus in mammals is a morphologically well-defined structure, and so are its main subdivisions. To define the homologous structure in other vertebrate clades, using these morphological criteria has been difficult, if not impossible, since the typical mammalian morphology is absent. Although there seems to be consensus that the most medial part of the pallium represents the hippocampus in all vertebrates, there is no consensus on whether all mammalian hippocampal subdivisions are present in the derivatives of the medial pallium in all vertebrate groups. The aim of this paper is to explore the potential relevance of connections to define the hippocampus across vertebrates, with a focus on mammals, reptiles, and birds.

Introduction

What can we learn from comparative studies of the hippocampus? An answer to this question is not straightforward since it depends, among others, on what one aims for. In this paper, we intend to define some basic requirements that need to be met before the question as such becomes tangible. For example, in order to compare, we need to define what to compare, and at which level of biological classification. Regarding the first, we will start with a definition of what to consider as hippocampus in the context of this paper, its divisions, and the possible relevant circuitry levels. As will become clear, decisions about definitions as well as about the level of biological classification, species, family, and order are strongly dependent on the available data. We will, therefore, use a pragmatic approach, restricting ourselves to available data relevant to the narrative of this paper. In this paper, we also aim to complement an accompanying paper [Butler, 2017] by emphasizing connectivity patterns as a tool to propose potential homology in the hippocampus.

Definition of the Hippocampus and Its Subdivisions

The first mentioning of the hippocampus in the mammalian brain can likely be found in the work of a pupil of the 16th century anatomist Vesalius, named Arantius [Lewis, 1923]. The first part of the term refers to part of the formation in mammals resembling a horse's head and the second part refers to the caterpillar, or "silkworm" appearance of the tail (for further details, see Butler [2017]). One of the first detailed and comparative studies on the

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structure and connectivity of the hippocampus is by Ramón y Cajal, published around the turn of the 19th century [Ramón y Cajal, 1893, 1911], followed by influential descriptions of the anatomy and connectivity of the main subdivisions of the hippocampus by his student Lorente de Nó [1933, 1934] and subsequent detailed studies from the 1960s and 1970s (for details, see Witter et al. [1989]). The typical hippocampus in mammals includes the dentate gyrus, the cornu ammonis (CA) fields CA1, CA2, and CA3, or hippocampus proper, and the subiculum. Although several authors have described an area CA4, we will not use this in the present paper and consider this part of area CA3. The hippocampus is a three-layered cortex, consisting of the molecular layer, directly deep to the pia, a cellular layer, and, deep to the latter, a polymorph layer. The superficial layer contains very few, mainly inhibitory neurons, and the polymorph layer has on average a larger number of neurons than the molecular layer. The neurons in the polymorph layer are either excitatory or inhibitory [van Strien et al., 2009].

Depending on the definition used, the entorhinal cortex (EC) is part of the hippocampus or part of the parahippocampal region. Here, we will take the perforant pathway, originating as the main cortical input from the EC to the hippocampus as belonging to the defining features of the main circuitry of the hippocampus (see also the next section). This is in line with the emphasis on the entorhinal-hippocampal connections, as mentioned by Ramón y Cajal [1902] already, based on his own work and referring to previously published data. In his seminal paper on the EC [Ramón y Cajal, 1902], he stated twice that the connections between the EC and the hippocampal formation are so conspicuous that they necessarily imply the functional solidarity of both centers. We will, however, not deal extensively with the comparative aspects of the EC in this paper (for more details, see Medina et al. [2017]).

The hippocampus is a key component of an ensemble of brain structures that became known as the limbic system. The term limbic is derived from an anatomical description by Thomas Willis [1664], who referred to the brain area that surrounds the brain stem as the limbus. Subsequently, Broca referred to the cortical fringe of the hemisphere, including the subcallosal, cingulate, and parahippocampal gyri as well as the underlying hippocampal formation, as "le grand lobe limbique" [Broca, 1878]. Although this designation was purely anatomical, Broca suggested that these limbic structures might constitute a functional entity. Much later, Papez [1937] postulated the presence of a closed circuit that would play an important role in the elaboration and the expression of emotions. This "Papez circuit" comprises a sequence of interconnected structures, i.e., the hippocampus projects by way of the fornix to the mammillary bodies which connect by way of the mammillothalamic tract to the anterior nuclei of the thalamus; from here, the cingulate cortex is reached, which through the ventral continuation of the cingular bundle is connected with areas in the parahippocampal region, including the EC, projecting back into the hippocampus. In 1952, MacLean [1952] coined the term "limbic system" suggesting that these structures, including the amygdaloid complex, represented the "visceral brain."

It was the seminal publication by Scoville and Milner [1957] that made the scientific community aware of the potentially important role of the hippocampus in episodic memory. In that paper, it was reported that bilateral removal of structures in the medial temporal lobe, including substantial parts of the hippocampus, the parahippocampal domain, and the amygdala, resulted in profound anterograde amnesia [Annese et al., 2014; Augustinack et al., 2014]. The implication of the hippocampus in memory processes boosted interest in its anatomical and functional organization. Major breakthrough findings, such as the discovery of long-term potentiation [Bliss and Lomo, 1973] as a potential synaptic mechanism for the formation and storage of memories, the discovery of place cells in the hippocampus [O'Keefe and Dostrovsky, 1971], and the subsequent influential theoretical description of the hippocampus as a cognitive map [O'Keefe and Nadel, 1978], strongly led the field into a focal research effort to unravel the mysteries of hippocampal circuits and functions. Interestingly, the idea of the hippocampus as part of a more elaborate network of limbic structures has started to make its comeback in recent years [Aggleton, 2014; Aggleton and Christiansen, 2015].

Standard Connectivity of the Hippocampus

The connectivity of the hippocampus known in that groundbreaking era was guided by two well-established conventions. First, the main fiber connection of the hippocampus was formed by the fornix, providing the output and input pathway of the hippocampus with subcortical structures like the septal complex and the mammillary bodies. Second, the EC provided the point of entry of cortical inputs to the hippocampus. This projection was initially referred to as the direct perforating spheno- or temporo-ammonic pathway by Ramón y Cajal [1893,

1902, 1911] as one of a tripartite connection system, collectively referred to as the temporo-ammonic pathway (for more details, see Stephan [1975]). The designation "direct perforating" referred to the massive entorhinal fiber bundles perforating the subiculum on their direct course into the hippocampus. This pathway became later known as the perforant pathway [Lorente de Nó, 1934]. An additional temporo-alvear tract was described as well, with fibers travelling from the EC through the alveus of the hippocampus into the CA fields. The third component, referred to as the angular pathway, carries mainly but not exclusively commissural fibers. As indicated by the name, in this early description, emphasis was on the projections to the CA fields, although projections to the dentate gyrus were included as part of the direct perforating temporo-ammonic/perforant pathway. In a detailed anterograde tracing description of the entorhinal-hippocampal connectivity in the rat in the mid-1970s [Steward, 1976], the projections to the dentate received more emphasis. The latter author referred to this projection as the temporo-dentate pathway, contrasting it with the temporo-ammonic pathway reaching the CA fields and the subiculum. Together with the knowledge about intrinsic hippocampal pathways, this led to the attractive concept of the so-called trisynaptic pathway as the blueprint circuit characterizing the hippocampus [Andersen et al., 1969; but see Amaral and Witter, 1989]. Over years, this also resulted in confusing changes in nomenclature such that the temporo-dentate pathway became erroneously referred to as the perforant pathway by many authors, since it perforated the hippocampal fissure on its way to the dentate gyrus, and the usage of temporo-ammonic pathway became restricted to the entorhinal projections to CA1. The trisynaptic circuit thus encompassed (1) the entorhinal, perforant pathway synapse on the dendrites of dentate granule cells, which in turn originate (2) the mossy fiber projection, synapsing onto the complex spines of the CA3 pyramidal cells. The latter originate not only the intrinsic auto-associative projections in CA3, but also (3) the Schaffer collateral projection, forming the third synapse on CA1 pyramidal neurons (Fig. 1a). In that concept, the projections from the EC to the CA fields became essentially ignored, and it was only in the late 1980s/early 1990s that they were "rediscovered" [Witter et al., 1988; Amaral and Witter, 1989; Yeckel and Berger, 1990] while the projections to the subiculum, also already mentioned by Ramón y Cajal, were introduced on the scene again [Witter and Groenewegen, 1990; Witter et al., 1992]. Since then, the projection to CA1 is referred to as the temporo-ammonic pathway by some, and by others as the direct EC to CA1 projection, forming one component of the perforant pathway. Within the context of the present comparative study, this vague nomenclature becomes a problem, since searching for the perforant path in a nonmammalian animal might become an issue, depending on how this pathway is defined. It is, therefore, appropriate to redefine the entorhinal-hippocampal projections, also because we now know that neurons in layer II are the main source of the entorhinal projections to the dentate gyrus and fields CA2 and CA3, and neurons in layer III give rise to the entorhinal projections to CA1 and subiculum (note that a small number of neurons in deeper entorhinal layers contribute to both projections). For the present paper, we, therefore, propose to differentiate between the EC layer II projection and the EC layer III projection. It has been shown that single layer II cells project to the dentate gyrus and CA2/CA3 [Tamamaki and Nojyo, 1993], but whether this is true for the layer III projection to CA1 and subiculum is as yet unclear.

Considering the fornix as the main if not sole hippocampal output pathway triggered a wave of experimental studies in which fornix lesions were considered as a convenient experimental model for the more complex hippocampal lesions. Although attractive, results from these studies rapidly pointed to a serious conceptual problem in that fornix lesions did not reliably mimic the profound amnesic syndrome seen after complete hippocampal lesions. In addition, the amnesic syndrome seen in a patient was characterized as anterograde amnesia, since memories from before the surgery seemed more or less intact, indicating that the actual memory storage had to be somewhere else in the brain, most likely in the cortex [Squire and Wixted, 2011]. Since the fornix does not provide an output pathway to the cortex, an emerging challenge was to find the potential pathway mediating memory storage in the cortex. This challenge was resolved by an insightful study in the rhesus monkey, published in a series of three papers showing that the subiculum projected to deep layers of the EC, which in turn contain neurons that are the origin of direct or indirect widespread projections to higher-order cortical areas [Van Hoesen and Pandya, 1975a, b; Van Hoesen et al., 1975; Rosene and Van Hoesen, 1977]. These findings were shortly after corroborated and extended in an extensive series of publications in the cat [Witter and Groenewegen, 1986], guinea pig [Sorensen, 1985], and rat [Kosel et al., 1982; Swanson and Kohler, 1986; Insausti et al., 1997]. These and subsequent studies painted the current more complex connectional diagram of the corticohippocampal system (Fig. 1b) [van Strien et al., 2009].

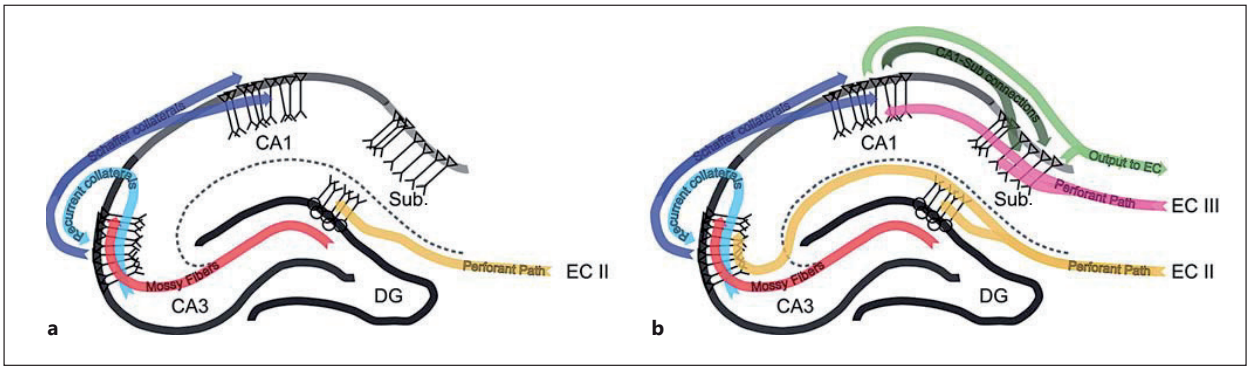

Fig. 1. Schematic representation of main hippocampal connectivity. **a** The traditional trisynaptic pathway comprising the entorhinal-dentate perforant path synapse, the dentate gyrus (DG)-cornu ammonis CA3 mossy fiber synapse, and the CA3-CA1 Schaffer synapse. Also indicated are the strong intrinsic CA3 auto-associational connections. **b** A more elaborate connectional diagram including the parallel entorhinal cortex (EC) layer II and III projections, as well as incorporating the subiculum and CA1 and subicular projections to the EC.

In our quest to find defining connections as arguments to establish homologies, we should, however, not ignore the massive fornix projection targeting a variety of basal forebrain and hypothalamic structures, including the lateral septum and to a lesser extent the medial septum, the nucleus accumbens, and several hypothalamic domains, with the mammillary bodies likely receiving the densest innervation [Kishi et al., 2000; Witter, 2006]. It is now well established that these pathways and the interconnected structures all play roles in higher-order cognitive functions [Aggleton et al., 2000], but manipulations result in dysfunctions dissimilar to those seen after damage to the corticohippocampal system. Clinically, the human syndrome of diencephalic amnesia is the closest to medial temporal lobe amnesia, and there is general agreement that all diencephalic patients share damage to the mammillothalamic tract [Van der Werf et al., 2003a, b; Aggleton et al., 2010]. A complete understanding of these complexities await further details about the connectivity and functional interactions of all structures involved.

Is the Characteristic Hippocampal Circuit Really Trisynaptic?

Aiming for solid features to embark on a comparative analysis, it is important to agree on what we are looking for to argue what in the nonmammalian brain might be the hippocampus. We could look for morphology, chemical or genetic identity of neurons, developmental origin, or aspects of circuitry. Focusing on the latter, in view of the above section on connectivity, is it the trisynaptic circuit that we should be looking for in nonmammalian species? In recent years, an alternative view has been proposed, which puts emphasis on the EC layer III projection and the marked reciprocating projections from CA1 and the subiculum. Reciprocity is a common feature of cortical connectivity, and the EC layer II projection is a clear exception to that common pattern in that neither the dentate gyrus nor CA3 seems to originate reciprocating projections to EC [van Strien et al., 2009]. So, it could be argued that searching for a canonical trisynaptic pathway might not be the best comparative approach. This line of thinking is supported by the suggestion that the dentate gyrus is unique to mammals [Striedter, 2016]. In this view, the medial cortex in reptiles and amphibians represents the pyramidal hippocampal layer, i.e., the CA fields, and no dentate granular cells are present. Per this scenario, the evolutionarily preserved circuit thus includes the pyramidal cells of the hippocampus, receiving cortical inputs from more lateral parts of the cortex, in turn sending output to the lateral cortex, and, via the fornix, to septum and hypothalamus.

The morphological definition of dentate granule cells as globular cells without basal dendrites is, however, ambiguous. In some mammalian species, such as postnatal rats, but also in adult monkeys and humans, dentate granular cells come in different forms, some being less globular, occasionally having basal dendrites, such they have some resemblance to pyramidal neurons [Treves et al.,

2008]. A better criterion might be that in the commonly studied mammals, the dentate granule cells give rise to a morphologically characteristic axon, the mossy fiber, showing complex, moss-like multisynaptic terminal complexes with equally complex spine structures on the target pyramidal neurons [Treves et al., 2008]. The mossy fiber projection also expresses high levels of zinc, as traditionally stained in several species with the Timm stain [Treves et al., 2008].

If the dentate gyrus is a mammalian addition, this raises the question what to do with the two components of the entorhinal inputs, the layer II versus layer III system. Since the layer II system projects to CA2 and CA3 as well as the dentate gyrus, would we expect that a similar division is already present in nonmammals? Alternatively, is the layer II projection an addition like the dentate gyrus, which subsequently expanded to also innervate adjacent pyramidals of CA fields? Assuming the latter, did this occur parallel to the dentate mossy fiber projection innervating CA3 and CA2 [Kohara et al., 2014; Haussler et al., 2016]?

Another recent hypothesis postulates that the dentate as such is not new, but that the folding of dentate, resulting in the hippocampal fissure and the discontinuity between dentate gyrus and CA fields, is the characteristic feature of the mammalian brain [Hevner, 2016]. Attractive as this may seem, this concept seems to pass over the fact that in all mammals studied, there are parts of the hippocampus that do not show these particular features. A good example can be found in the brain of marsupials, such as the opossum (Fig. 2). Whereas at more posterior levels (Fig. 2a2, 3), the hippocampus indeed comprises a folded dentate gyrus and a separated CA field emerging close to the hilar region of the dentate, at more anterior levels (Fig. 2a1), the two structures become aligned such as to show a striking similarity to what is found in some reptilian species [Striedter, 2016; Butler, 2017]. A similar arrangement has been described in monotremes, such as Echidna [Hassiotis et al., 2004]. However, also in placental mammals, such a nondifferentiated hippocampal-like structure is present, called the taenia tecta (Fig. 2b) and the supracallosal indusium griseum [Stephan, 1975; Treves et al., 2008].

Subdivisions and Standard Connectivity Compared

The cortex of reptiles comes in different flavors, one main group, including lizards and snakes, presenting a marked three-layered cortex, while the others come with variable changes in that pattern from a less laminated version, generally seen in turtles to the one in crocodiles, that comes closer to the totally nonlaminated version seen in birds [Striedter, 2016]. In lizards, the medial part of the cortical sheet is commonly divided into three domains, a small-celled medial domain, a large-celled mediodorsal domain, continuing into the dorsal cortex, which is bordered in turn by the lateral cortex. The small-celled medial domain contains several morphologically different cell types, some of which give rise to a zinc-positive mossy fiber-like projection to the adjacent large-celled mediodorsal and dorsal domains, indicative for a dentate homologue. Zinc-positive terminals have also been reported on neurons in the polymorph layer of the small-celled portion. The targets are large neurons looking similar to hilar mossy cells described in the mammalian hippocampus [Treves et al., 2008]. These observations seem to indicate that, at least in some reptiles, a dentate-like structure is present, not well differentiated from the adjacent cortex which could be considered to represent an as yet not differentiated representation of the CA component described in mammals. From a morphological point of view, the resemblance between the small- and large-celled parts of the lizard cortex to what has been described for the taenia tecta and indusium griseum in rodents is striking, including a zinc-positive projection system that has been described in mice [Adamek et al., 1984; Laplante et al., 2013]. These authors conclude that the indusium griseum and potentially also the taenia tecta might be phylogenetically old representations of the hippocampus. However, studies in the Madagascan hedgehog tenrec led to the conclusion that the indusium griseum, again showing a zinc-positive projection, might be correlated with lizard medial cortex, but that it is incorrectly considered a hippocampal homologue [Kunzle, 2004]. One would hope that more detailed functional studies on the lizard brain as well as on the taenia tecta and indusium griseum in mammals might clarify the validity of these claims.

Alternative Subdivisions of the Hippocampus

Alternative ways to divide the hippocampus have been proposed, contrasting to the trisynaptic and EC layer II versus layer III partitions. Among the most prominent ones are a functional differentiation along the longitudinal axis, and a functional differentiation represented by two parallel cortical input/output systems, mediated by different components of the EC.

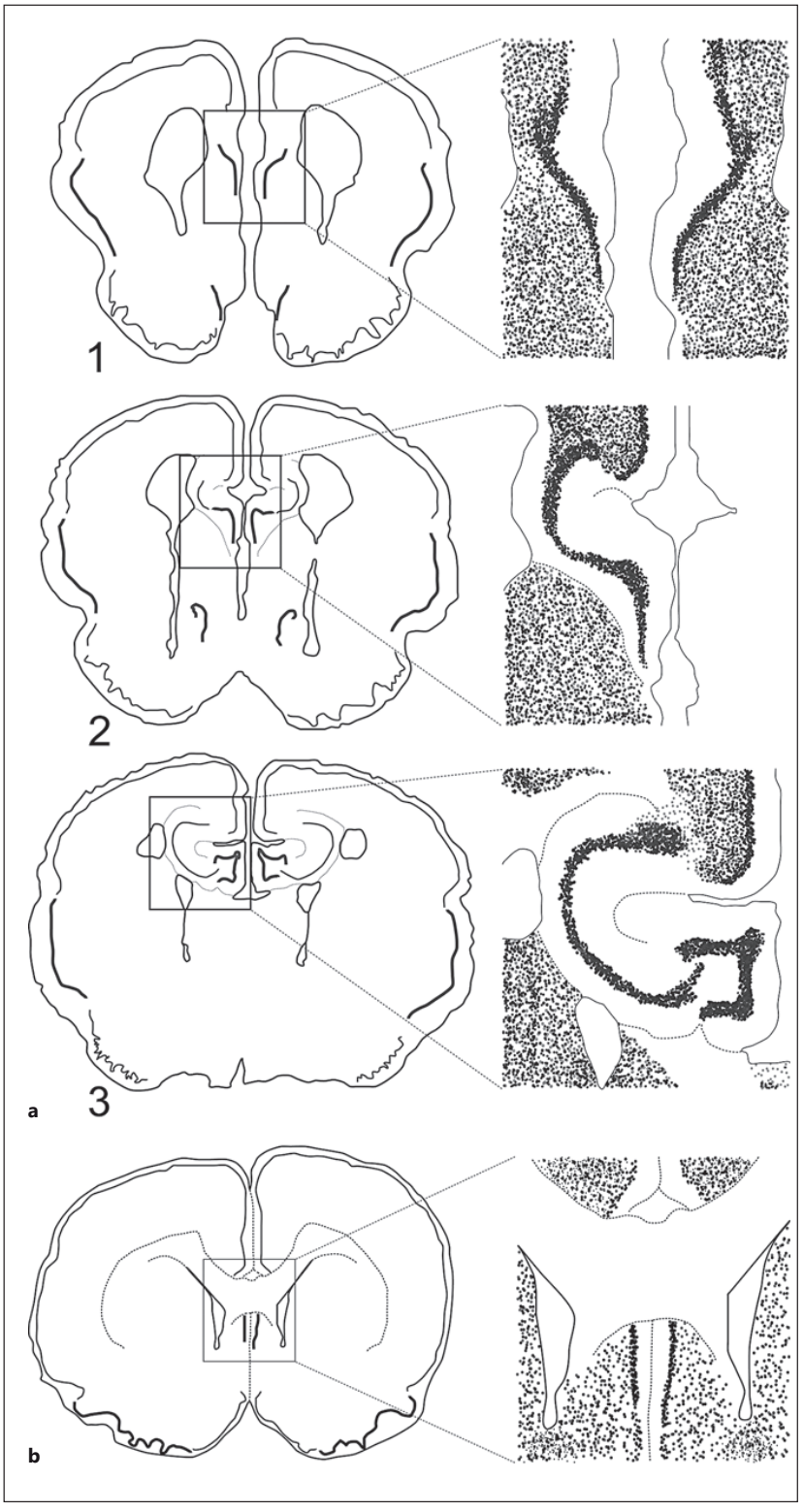

Fig. 2. Presence of an unfolded dentate gyrus in nonplacental and placental species. **a** Series of coronal sections from rostral (1) to caudal (3) through the brain of the marsupial opossum. At most anterior levels (1), the hippocampus/dentate gyrus exhibits a nonfolded appearance, comparable to the medial cortex in reptiles. **b** In rodents, such as the rat, a comparable nonfolded structure, called the taenia tecta, can be found at levels ventral to the genu of the corpus callosum.

The Hippocampal Long Axis

Based on a large body of connectional and functional data in rodents, carnivores, and primates, a dominant view has been that the dorsal (or posterior) hippocampus is implicated in memory and spatial navigation, and the ventral (or anterior) hippocampus mediates emotional, anxiety, and stress-related behaviors. Interestingly, such a functional differentiation might exist in the avian hippocampus as well [Smulders, 2017]. The border between the two domains in the mammalian hippocampus has not been well established, and some authors have suggested dividing the hippocampus into three components, inserting an intermediate domain. Gene expression studies demonstrate multiple domains along the hippocampal long axis, which often exhibit sharply demarcated borders. Together these data suggest a model in which long-axis gradients are superimposed on discrete connectionally and genetically defined domains, resulting in at least three functionally different domains [Strange et al., 2014; Maass et al., 2015; Navarro Schroder et al., 2015].

A striking example of functional differences along the long axis has been reported with respect to the representation of space through the firing properties of place cells, found in all of the CA divisions [O'Keefe and Dostrovsky, 1971; O'Keefe, 1976; Lu et al., 2015], but also to a lesser extent in dentate gyrus and the subiculum. The most detailed analysis has been carried out in CA1 and CA3, showing that the size of a place field is related to the position of the place cells along the long axis. Place cells in the dorsal hippocampus have the smallest place fields, and, at more ventral levels, the sizes increase gradually [Kjelstrup et al., 2008]. Place field size can be interpreted as a measure of spatial scale, indicating that environments might be represented at different spatial resolutions along the long axis of the hippocampal formation. Recent functional MRI findings in humans support such a difference in representational resolution along the hippocampal long axis [Evensmoen et al., 2013, 2015]. These findings, when combined with the comparative data summarized above, lead to a clear prediction about a possible spatial code in, for example, the medial and dorsal cortex of the lizard. In case place cells were to be found in this cortical domain, they will show a gradient such that spatial representation anteriorly is more fine grained than at more posterior levels. This might result in functional differences in the medial cortex, as suggested previously [Hoogland et al., 1994]. This prediction will hold irrespective of whether the lizard medial cortex comprises a dentate gyrus and an EC layer II input system or not, since place fields in rodents are independent of the EC layer II-dentate-CA3 system [Brun et al., 2002]. Instead, they depend on the EC layer III system, more in particular the component that arises from the more posteromedial part of the EC [Brun et al., 2008]. Moreover, this input plays a role in long-term spatial memory [Remondes and Schuman, 2004].

Parallel Cortical Pathways

The second differentiation is strongly based on observations that the more posteromedial part of the EC, generally referred to as the medial EC (MEC), has been shown to be functionally and connectionally different from the anterolateral part, the so-called lateral EC (LEC). Firing of neurons in the MEC represents spatial, directional and speed information [Fyhn et al., 2004; Sargolini et al., 2006; Solstad et al., 2008; Kropff et al., 2015]. In contrast, recordings in LEC have not indicated the presence of pure spatially modulated neurons; rather the firing of neurons in LEC seems to reflect the presence of objects in context [Tsao et al., 2013; Knierim et al., 2014]. Although the causes for these striking functional differences are as yet not fully understood, it is likely that different connectional streams into MEC and LEC, strongly involving different connectivity patterns from adjacent parts of the parahippocampal regions such as the postrhinal/parahippocampal cortex and perirhinal cortex, are key determinants of this difference [Witter et al., 2000; Eichenbaum et al., 2012; Ranganath and Ritchey, 2012]. Interestingly, the projections of the two entorhinal domains to area CA1 and the subiculum in all mammalian species studied, including nonprimates and primates, are topographically organized along the transverse axis of both fields [Witter and Amaral, 1991; Witter et al., 2000; van Strien et al., 2009]. Recent connectional MRI studies in humans have pointed to a similar connectional bipartite system separating anterolateral from posteromedial EC, showing clear differences with respect to connectivity measures in the hippocampus, resembling those reported in rodents [Maass et al., 2015]. This thus indicates that functionally different types of input may be mapped onto different hippocampal domains along the transverse axis, a prediction that was shown to be correct in CA1 in rats with respect to spatial information carried by firing properties of neurons [Henriksen et al., 2010]. It remains to be established whether comparable functional differences exist in other clades, but recent gene expression patterns during embryological development indicate that in birds and lizards, LEC and MEC might be identifiable [Abellan et al., 2014; Medina et al., 2017]. Whether these different entorhinal domains in birds and lizards show connectional

differences, comparable to those seen in mammals, is open to further study. It is of interest that in the lizard *Gekko gecko*, two connectional pathways have been described originating from lateral and medial portions, though only the lateral portion seems to project to the small- and large-celled medial cortex [Hoogland and Vermeulen-Vanderzee, 1995].

Concluding Remarks

For all mammalian species where we have connectional and functional data, it is apparent that the hippocampus receives its main cortical inputs from the EC, organized in a 2 × 2 matrix of origin, consisting of EC layer II and III projections on one axis, and the LEC and MEC on the other. This matrix of connections seems well conserved. With respect to reptiles, most data on the potential homologous areas in the medial and lateral cortex are restricted to a few species of lizards, and although genetically defined LEC and MEC might exist, data on the connectivity of these recently identified areas are sparse if not missing. In birds, the situation is even less clear although, at least in the chicken, comparable entorhinal areas have been identified.

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Spatial representation in the hippocampal formation: a history

Edvard I Moser, May-Britt Moser & Bruce L McNaughton

Since the first place cell was recorded and the cognitive-map theory was subsequently formulated, investigation of spatial representation in the hippocampal formation has evolved in stages. Early studies sought to verify the spatial nature of place cell activity and determine its sensory origin. A new epoch started with the discovery of head direction cells and the realization of the importance of angular and linear movement-integration in generating spatial maps. A third epoch began when investigators turned their attention to the entorhinal cortex, which led to the discovery of grid cells and border cells. This review will show how ideas about integration of self-motion cues have shaped our understanding of spatial representation in hippocampal–entorhinal systems from the 1970s until today. It is now possible to investigate how specialized cell types of these systems work together, and spatial mapping may become one of the first cognitive functions to be understood in mechanistic detail.

Although the study of the cellular and circuit mechanisms of spatial representation in the brain today is centered on the hippocampal and parahippocampal formation, the study of spatial coding did not begin there, but rather began with the parietal cortex, in the form of early observations on patients with parietal damage[1,2]; in many respects, one takes a risk in attempting to limit the discussion to the hippocampal formation[3]. Nevertheless, in studies of spatial coding, some of the most 'paradigm-shifting' discoveries and ideas have come from recordings within the greater network of the hippocampal formation, particularly the dorsal parts of hippocampus, entorhinal cortex, presubiculum, and parasubiculum, where cells exhibit place-dependent activity independently of the animal's behavior or the task that it is performing (**Fig. 1**). Key among these insights were the discoveries of place cells (**Fig. 2**)[4], head direction cells (**Fig. 3**)[5–7], and grid cells[8,9], each of which represent quantum jumps in our understanding that there is a system in the brain that has evolved to produce a representation manifold that can be linked to position (grid cells), an inertial compass (head direction cells), and a system for mapping external features and events onto internal and, at least locally, metric coordinates (place cells). In broad terms, these components and their interactions were predicted by O'Keefe in 1976 (ref. 10).

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Also key to the emergence of a model for spatial representation was a gradual understanding of the role played by different spatial reference frames and their interactions. Space can be represented in three reference frames: egocentric (defined in relation to a body part axis), allocentric (based on spatial relationships to or among external features), and inertial or idiothetic (relative location and orientation based on direction and distance moved from an arbitrary reference point). Navigation in an idiothetic reference frame is often referred to as 'path integration', a process by which animals use self-motion cues (such as motor efference, optical flow, and vestibular information) to keep track of their own location relative to a starting point[11–14]. Decades of investigation have shown that egocentric space is not represented primarily in the hippocampal formation but rather in parietal cortex and associated regions[15–17]. O'Keefe's studies showed from the outset that, instead, place cells encode an animal's location in an orientation-independent reference frame[10]. Although the term allocentric was applied to place cell representations, O'Keefe recognized early on that these representations may rely "on the fact that information about changes in position and direction in space could be calculated from the animal's movements."[10] Yet it was not until the discovery of head direction cells in the 1980s[5–7] and the realization that these cells were indeed performing integration of head angular velocity[18] that the concept emerged, in the 1990s, that the entire hippocampal formation might be using an idiothetic reference frame—or path integration—as a basis for its coordinate system[19]. The possibility of a path-integration mechanism outside the hippocampus proper[3,20,21] was reinforced at this time by studies showing that, unlike place cells, spatially modulated cells in the entorhinal cortex and subiculum had environment-independent spatial firing patterns[22,23]. Today it is generally recognized that path integration plays a fundamental role in spatial coding in the hippocampal formation, although there continues to be controversy as to whether path integration is the primary determinant of place cell and grid cell firing or whether it plays an equal or subordinate role to the integration of information from external stimuli[24–26].

Finally, a discussion of model shifts would not be complete without some realization of the role that technology has played (**Fig. 4**).

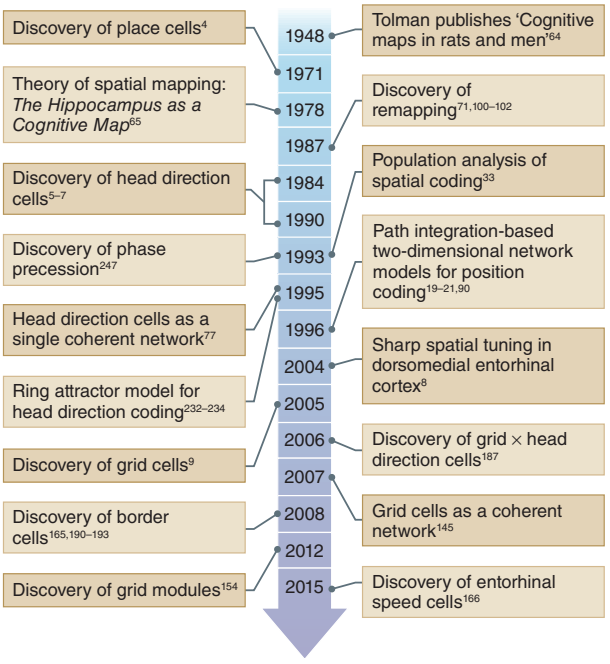


Figure 1 Selection of historical milestones in the study of spatial coding in the hippocampal formation.

Key technical advances have been the shift from recording single cells in restrained, usually anesthetized, animals to recording in freely behaving ones[4,27–29]; the development of quantitative video-tracking methods for rodents during hippocampal recording experiments[30,31]; the invention of stereo (tetrode) recording[32] (**Fig. 4a**) and its extension to large neuronal ensembles[33] (**Fig. 4b–d**); the development of micromachined silicon electrode arrays[34]; new cell-type-specific optical and chemical methods for stimulation[35–37]; and, most recently, the development of large-scale Ca^{2+} cellular imaging in both freely moving animals[38] and in restrained animals locomoting in virtual reality environments[39,40]. The importance of recording from substantial numbers of cells in interpreting coding dynamics for the hippocampus or any other neural system cannot be overemphasized. Apart from the obvious computational and statistical analysis power enabled by collecting data from large numbers of simultaneously active neurons, it is clear that many results that we now understand as across-trial variations in population dynamics may have been attributed to differences in single neuron classes in early single-neuron recording studies.

We have taken on the task of trying to present, in a relatively small space, an historical overview of some of the paradigm-shifting developments that led to our current understanding of spatial coding in the hippocampal formation. This task is daunting for several reasons, not the least of which is that the number of important experimental and theoretical contributions has risen (and continues to rise) almost exponentially since 1971, when O'Keefe and Dostrovsky, after recording in freely behaving rats from what today would be considered a very small sample of CA1 units, made the bold claim that the hippocampus might construct a spatial map[4] (**Fig. 2**). Length restrictions have forced us to focus the review on one particular set of ideas that has inspired the investigation of hippocampal representations of space almost since the beginning of studies of place cells, namely that spatially localized firing to a large extent reflects the dynamic integration of self-motion—or path integration—as animals move around in the environment. We shall demonstrate how the idea of a path-integration input explains many fundamental properties of place cells and how this, in turn, led investigators in the single-cell recording field to identify a path-integration-dependent neural system consisting of multiple functionally specialized cell types in the parahippocampal cortices.

We shall demonstrate that path integration appears as a leitmotif that follows the history of spatial representation in the hippocampal formation across generations of investigators. Yet by directing our spotlight to path integration, we are forced to leave out contributions and research directions that have contributed critically to the broader understanding of place cells and hippocampal systems function, beyond the representation of self-location. First of all, the more than four decades of hippocampal spatial mapping studies have developed alongside an equally productive line of investigations, using a variety of methodological approaches, into the basis of memory in the same brain system[3,41–47]. The focus of this review is on the coding of space, but, as we will acknowledge, this does not rule out a broader participation of hippocampal neurons and place cells in representation of experience[48–50]. In shying away from the memory functions of the hippocampus, we shall also pass over the vast and growing literature on how replay and preplay of firing sequences may enable consolidation and storage of hippocampal memory through interactions with neocortical neural networks[51–54], and we shall not discuss the important but separate question of whether or how place cells are used for goal-directed navigation and route planning[55–59]. We have also left out dozens of pioneering studies of temporal coding and network oscillations, including theta rhythms, that have shaped our current understanding of hippocampal function beyond the representation of space[49,60–62]. Finally, this review is dominated by work in rats and mice, reflecting the use of freely moving rodents as subjects in nearly all studies of spatially modulated cells in the hippocampal formation (see **Box 1** for extensions to the primate brain).

The origin of the spatial signal

In 1971, O'Keefe and Dostrovsky observed that neurons in the rat hippocampus had what appeared to be spatial receptive fields[4] (**Fig. 2a,b**). In their 1971 paper, the number of place cells and evidence for localized firing was limited, but much more substantial data were presented by O'Keefe in 1976 (ref. 10). By this time, after thorough study of hippocampal activity in unrestrained rats[29], Ranck had also seen place cells[63]. The O'Keefe paper showed that place cells fired whenever the rat was in a certain location in the local environment. Different cells had different place fields, such that at all locations investigated in the hippocampus, the animal's location could, in principle, be inferred from the joint activity of a fairly small sample of neurons[10] (for direct demonstration, see ref. 33 and **Fig. 4c,d**). Based on this observation and inspired by Tolman's proposal that navigation is guided by internal cognitive maps[64], O'Keefe and Nadel[65] suggested that place cells are the basic element

of a distributed allocentric cognitive map of the animal's environment (**Fig. 2c**). The spatial relations between landmarks provided by this map were thought to enable animals to find their way independently of local view or movement trajectories, using what O'Keefe and Nadel called a locale strategy. This contrasted with route strategies, which do not take into account the relationship between landmarks. The latter strategies included a spectrum of routines from simple beacon navigation to more complex action sequences. O'Keefe and Nadel's proposal represented a major landmark in the conceptualization of hippocampal function. Their book, *The Hippocampus as a Cognitive Map*, synthesized and reinterpreted decades of discordant experimental studies using a range of experimental approaches, particularly lesions, and put these studies into a coherent theoretical framework organized around the concept of place cells as the cellular basis for representation of space as well as events and experiences associated with space. The book proposed a neural implementation of Tolman's concept of the cognitive map, with visionary perspectives on how such a map might enable a breadth of cognitive functions in higher species, including humans. Today, 40 years after its publication, *The Hippocampus as a Cognitive Map* remains the theoretical pillar on which nearly all subsequent study of spatial coding in the hippocampal formation rests.

The early years of research on place cells, in the late 1970s and 1980s, were dominated by attempts to prove that the place signal was indeed spatial and, given this, to understand what caused place cells to fire where they did, based on the idea that it was some constellation of external sensory cues, rather than a single cue or some other cause (for example, ref. 66). Two salient observations in this period that both advanced knowledge and increased perplexity were the findings that place cells appeared to be completely direction-dependent when animals ran repeatedly on restricted paths[30] but were unaffected by head direction during free foraging in a large cylinder[67]. Perplexity about the mechanism of place cells was further increased by the fact that place cells had a sort of 'memory': they rotated their fields when external cues were rotated but continued to fire in relation to the last-seen cue location when the cues were removed[68,69]. Indeed, early studies indicated not only that place cells continued to fire in the 'correct' location in total darkness but also that fields could be formed when animals were introduced to an environment in darkness and were minimally affected when the lights were subsequently turned on[70]. Nevertheless, place fields became linked to external cues and rotated to maintain registration with them when the cues were rotated between sessions[68,71].

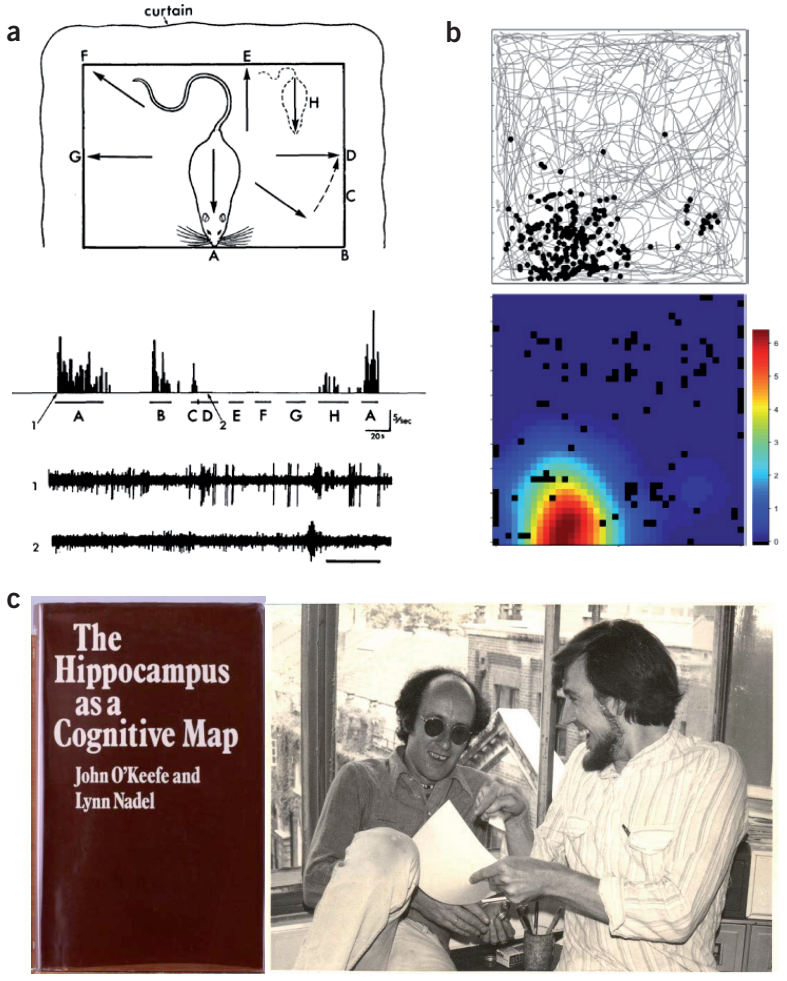


Figure 2 Place cells. (**a**) First place cell described[4]. Arrows and letters mark positions at which the animal was restrained as it was pushed or coaxed around the test platform. Firing rate of the unit is illustrated by the frequency histograms in the middle of the figure. Letters correspond to positions, and lines indicate periods of restraint. Bottom lines show spikes at the onset of the unit response at A (1) and during the absence of a response at D (2). Calibration bar, 400 ms. Note that the cell responds selectively at only a few positions. O'Keefe and Dostrovsky reported 8 units of 76 recorded hippocampal cells that responded solely or maximally when the rat was situated in a particular part of the testing platform and facing in a particular direction. Note that the single-electrode technology available to the authors at the time likely precluded regular good isolation of cells, which may have limited the number of clear 'place' responses observed. (**b**) A place field as typically displayed today. Top: rat's trajectory in gray; spike locations superimposed as black dots. Bottom: color-coded rate map; dark red is maximum rate; blue is silence. Regions not visited in black. (**c**) Left: the book by John O'Keefe and Lynn Nadel was long a 'bible' in the study of spatial coding in the hippocampal formation. Right: Nadel (left) and O'Keefe (right) during preparation of the book. Photo taken by Dulcie Conway around 1975, reproduced here courtesy of John O'Keefe[264]. Panel **a** reproduced with permission from ref. 4, Elsevier.

The foregoing studies were soon followed by a number of observations that cast further doubt on the external sensory origin of place fields: most place fields had asymmetric firing fields in an environment with a symmetric cue configuration[72]; place fields could dynamically shift between a reference frame defined by a reward box that moved relative to the laboratory reference frame and the lab reference frame itself[73,74]; the location and orientation of place fields followed the rat when the rat was rotated independently of the environment[75,76]; place cells and head direction cells exhibited coordinated drift error in a cylindrical environment[77,78]; the size of place fields was almost completely independent of local cue density, spatial frequency, or

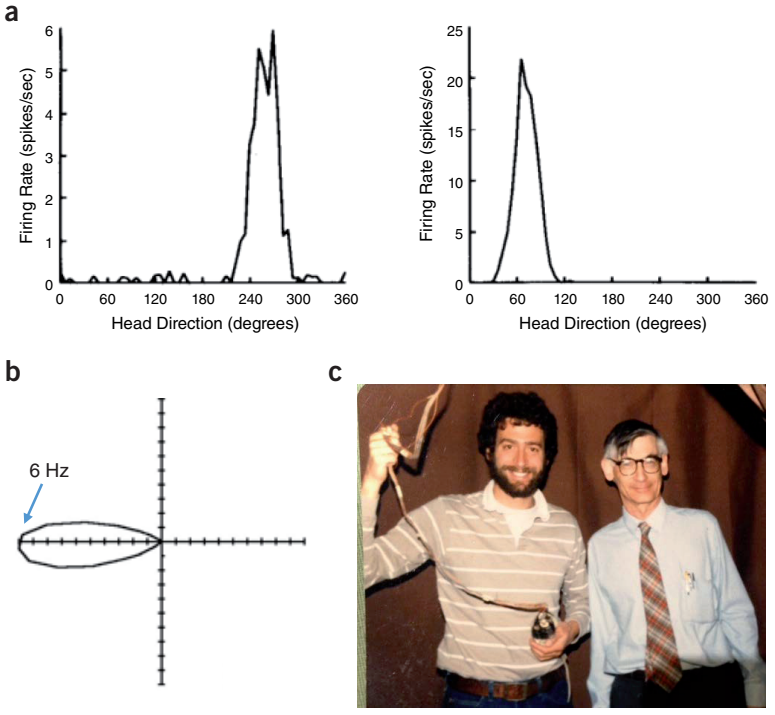


Figure 3 Head direction cells[6]. (**a**) Firing rate as a function of head direction for two representative cells from two different animals. (**b**) A head direction cell firing rate in polar coordinates. Peak firing rate, in the left orientation, is 6 Hz. (**c**) Jeffrey Taube (left) and James B. Ranck Jr. (right), at SUNY Downstate Medical Center in Brooklyn., N.Y., in 1987. Photo courtesy of Jeffrey Taube. Panel **a** reproduced with permission from ref. 6, "Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis," J.S. Taube, R.U. Muller & J.B. Ranck Jr., 1990, in *Journal of Neuroscience*, Vol. 10, pages 420–435.

salience[79] but varied systematically along the septotemporal axis of the hippocampus[80,81]; in rats with age-related memory impairment[82] or with NMDA receptors blocked[83], place fields appeared perfectly normal in a novel environment but could be completely rearranged when the animals were returned to the same environment after even a short delay; the place field map as a whole dynamically expanded when motor and vestibular information about movement speed was disrupted, in the absence of changes in landmark inputs[84]; place cells shut off completely when animals were restrained from locomotion[85]; and finally, the variation in scale of place fields along the hippocampal septotemporal axis was strongly correlated with the gain of physiological speed signals[86].

In spite of gradually accumulating evidence for an, in many ways, nonsensory origin of spatial receptive fields in the hippocampus, the lack of proper quantification prevented a general acceptance of this idea, and much of the initial effort was thus spent on proving that the signal was indeed spatial. As this skepticism was gradually overcome, investigators began to focus on how place cells might be synthesized as higher-order integrators of sensory data, perhaps endowed with memory properties. However, this sensory-integration approach changed, literally overnight, when James Ranck brought a video of a recorded head direction cell to the 1984 Society for Neuroscience meeting[87] (**Fig. 3**). Head direction cells are cells that fire specifically when the animal faces a certain direction[5–7] (**Fig. 3a**,**b**). Ranck first encountered these cells in the dorsal presubiculum—almost by accident, in an experiment in which electrodes targeted to the subiculum went astray[87]—but they were later observed across a wide network of cortical and subcortical regions[88,89]. In the same way that place cells covered all locations of an environment, the preferred firing directions of head direction cells were distributed evenly around angular space, enabling precise read-out of head direction in neural networks downstream of head direction cells. If the brain was endowed so clearly with an internal compass, as suggested by Ranck's 1984 movie, the idea that it also had a map became much more palatable. However, the first full publication on the basic properties of head direction cells did not appear until 1990, in joint work by Ranck, Taube, and Muller[6,7]. By that time, it was already recognized that the basis of the head direction signal was likely integration of head angular velocity, and the outline of a model for how this integration was performed using conjunctive head direction × head angular velocity cells (observed in dorsal presubiculum and parietal cortex) was proposed[18].

To many investigators, the foregoing observations collectively pointed almost inescapably to the hypothesis that the primary determinant of the cognitive map is some form of coordinate system in which head angular velocity and linear velocity are integrated over time to express displacement and orientation from a starting point (path integration)[19–21,90,91] (**Fig. 5**). According to this view, the path-integration mechanism assigns place fields based on motion integration. In the absence of external stationary input, errors from noise in the self-motion integration process accumulate, and place fields (and head direction tuning curves) would start to drift. However, in environments with salient cues, rapidly formed associations between cues and place cells enable stabilization of the firing fields, and previously formed maps can be recalled from session to session[10,19–21,90], possibly cued by landmark information conveyed through the dorsal presubiculum[92]. Nevertheless, there is also some support for the idea that place cells are formed by integration of salient sensory inputs, independently of movement. One of the main observations presented in favor of this concept is that place fields could be seen to expand[71] or stretch[93] in response to corresponding distortions of the enclosure in which recordings took place. However, such distortions do not occur when the animal is introduced *ab initio* into the distorted environment, only when the animal has first experienced the undistorted version. Stretching or expanding can thus be seen as a result of the external inputs attempting to correct the path integrator based on prior associations[90].

During the past decade, virtual environments have enabled investigators to dissociate with increased rigor the relative contributions of self-motion inputs and stationary landmarks. Typically, head-fixed mice or rats run on an air-cushioned ball or a circular treadmill while visual flow is projected onto an immersive screen at a rate that directly reflects the animal's running speed and direction, emulating the sensory-motor coupling of the real world[39,40]. When the virtual environment is linear, as on a treadmill, hippocampal place cells exhibit firing fields that depend on distance moved[94,95] or

stationary cues on the screen[94], with some variation between cells[94]. Reducing the gain of ball-to-virtual-scene movement causes place fields to move toward the start of the virtual track, as expected if firing locations are determined by self-motion, but the shift is generally smaller than expected from movement distance alone, pointing to an additional role for visual inputs[94]. The dual dependence on self-motion cues and external cues confirms earlier studies in which these sets of inputs were disentangled in real environments[73,74,93]. However, when the virtual environment is made two-dimensional and movement of the head remains restricted, localized firing breaks down, although a small influence of distance traveled is detectable[96]. In contrast, when body and head rotation is unconstrained, stable position coding persists[97]. Together these studies point to vestibular signals (which are impoverished during head fixation) as a critical source for integrating velocity and direction signals into a coherent two-dimensional representation, in agreement with earlier work showing that place fields are disrupted following inactivation or lesions of the vestibular system[98,99].

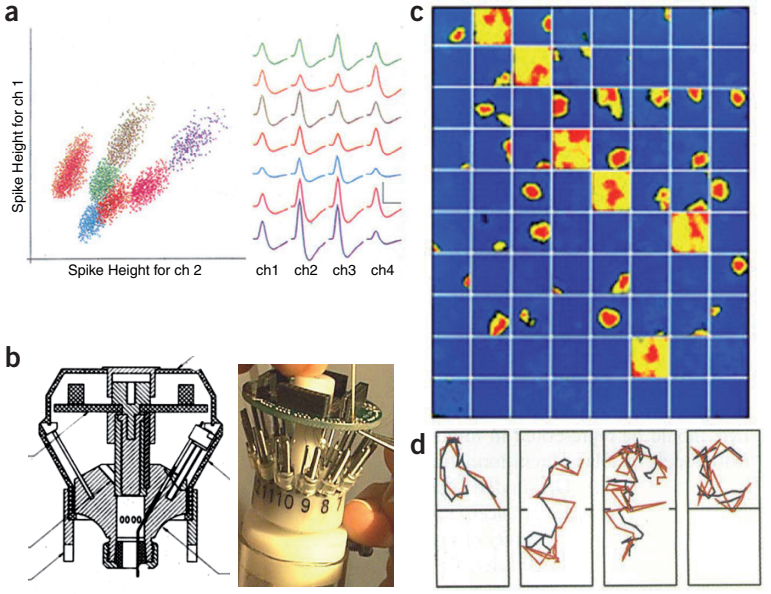


Figure 4 Ensemble recording technology. (**a**) The principle of tetrode recording proposed by McNaughton *et al.*[32] exploits the variation in extracellular spike height as a function of distance to the recording site to resolve multiple single units in structures such as hippocampus, where the neurons are fairly tightly packed. Example of spike amplitude clusters from a tetrode recording showing two of the four spike-amplitude dimensions. The corresponding spike waveforms are shown on the right. (**b**) A 48-channel, 12-tetrode probe array (hyperdrive) from ca. 1995. This system exploited the flexibility of wire tetrodes, which allowed researchers to advance them by pushing them through gently curving tubes (like a mosquito proboscis). (**c**) Multitetrode recording made it possible to record from more than 100 hippocampal neurons simultaneously. Here we show 80 firing rate maps from simultaneously recorded CA1 cells as the rat ran in a 70 × 70-cm arena[33]. Firing rate is color-coded from blue (silent) to red (maximum rate). Note that many CA1 cells were virtually silent in this particular arena, whereas about 40% had place fields. Six of the recorded cells correspond to fast-spiking cells (interneurons), which have much less spatial selectivity. (**d**) Examples of the actual (blue) spatial trajectory of the rat and the trajectory reconstructed from the population firing-rate vector (red). Panel **a** reproduced with permission from ref. 80, "Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat," M.W. Jung, S.I. Wiener & B.L. McNaughton, 1994, in *Journal of Neuroscience*, Vol. 14, page 7347–7356. Panels **c** and **d** reproduced with permission from ref. 33, AAAS.

Remapping: global, partial, local, and rate

In the late 1980s, Muller and Kubie began a series of investigations on the effects of changing the most salient visual cues in a cylindrical environment and introducing various local cues[71,72,100–102] (**Fig. 6**). As alluded to above, cue-card rotations, changes in the size or color of the cue card, or even removal of the cue card altogether rarely changed the radial coordinate of the field but could change the angular coordinate, completely unpredictably in the case of complete removal of the cue card when the rat was not present (**Fig. 6b**). They coined the term 'remapping' to describe any manipulation-induced changes in the firing of place cells. These could include mild changes in the firing characteristics in a few cells, such as when new objects or walls were placed in a cell's place field, up to radical changes in the location of firing, including the disappearance of a field altogether, which was sometimes observed when the environmental shape was changed or visual cues substantially altered.

Whether sets of place cells remapped completely or only partially depended on the experimental conditions. The terms 'global', 'partial', and 'local' remapping were introduced by Knierim and McNaughton[103] in an attempt to distinguish situations in which only fields near a specific, manipulated cue changed from situations in which there was a general (partial or complete) rearrangement of fields throughout the environment. Such limited remapping is often seen when the animal is placed in nonuniform environments[104,105] or in cases of deficient plasticity as discussed above[82,83]. The concept of remapping was clarified considerably by several experiments that followed. In 2005, Leutgeb *et al.* showed that, when the cues in the recording chamber or its shape were radically changed between sessions that took place in the same physical location, CA1 and CA3 place cells underwent substantial changes in their firing rates, without changing their firing locations[106] (**Fig. 6c**). These changes could be sufficient to make a field appear to be present in only one condition, unless the rate map graphs were rescaled. In contrast, when the recordings took place in identical apparatus located in two separate rooms, the place field distributions became completely uncorrelated. Leutgeb *et al.* made the distinction between 'rate remapping' for the former situation and 'global remapping' for the latter. Thus, it appears that, under conditions in which the path-integrator coordinates likely remain consistent, changes in external input or, indeed, internal variables such as motivation, working memory, or action plans, can result in dramatic changes in firing rate while firing location remains unaltered[107–110]. Leutgeb *et al.* suggested that rate remapping might be the cause of apparent partial remapping or direction dependency on linear tracks. The role of the path-integrator coordinates in governing rate versus global remapping was fairly decisively demonstrated by Colgin *et al.*[111], who showed that when environmental shape was gradually morphed between a circle and a square, abrupt, global remapping only occurred if the rats had previously been allowed to locomote between a circle and a square via a connecting tunnel. When rats were pretrained on the two shapes in the same location, only rate remapping was observed. Thus, it was the path integrator that determined whether global or rate remapping was observed.

The presence of a nonspatial code on top of the place code (rate remapping) is consistent with dozens of studies, starting in the 1980s, showing that place cells encode more than space. Cells with clear place fields in one task were shown in other tasks to respond in a time-locked manner to various nonspatial features of the environment or the experience, such as odors[112–114], textures[115], conditioned tones[28,116,117], or temporal stages of the experiment[118]. However, in combination with the remapping studies, these observations suggest that hippocampal cells respond conjunctively to spatial and nonspatial variables, with the latter represented as changes in the rate distribution. Experience-related changes in rate distribution can also account for moment-to-moment variability of firing rates within place fields (overdispersion)[119]. The conjunctive nature of spatial and event-related firing is demonstrated elegantly in a more recent study of hippocampal activity after systematic variation of location, food cups (objects), and color or pattern of the recording box (context)[120]. The majority of cells in this study fired at specific locations but with rates depending on context and objects. Thus, when location is clamped, unique constellations of cues give rise to unique rate patterns, implying that each experience is characterized by its own hippocampal–neocortical output, even when those experiences occur at a fixed location. This uniqueness is a necessary condition for the widely held view that hippocampus may provide an index that links memory attributes distributed widely over neocortex[121–123]. The wide range of stimulus configurations that activate hippocampal firing, over and above space, has been taken as evidence for a broad involvement of the hippocampus in episodic memory, where space is just one of several attributes of the encoded representation[48].

Lest one conclude from the foregoing that the phenomenon of remapping or the necessity or dominance of path integration is now fully understood, it is necessary to consider some remaining flies in the ointment. First, Tanila, Shapiro, and Eichenbaum[124,125], and later Knierim[126], have shown that, when an animal is highly familiar with the local and distal cues in an environment, rotating these cue sets relative to each other can cause some CA1 cells to follow the local set while others simultaneously follow the distal set (still others may remap). Such discordant responses are stronger in CA1 than CA3 (ref. 127). These effects are not inconsistent with a path-integration-based origin of the place fields, if one assumes that the subsequent, plasticity-dependent association between cues and place cells that leads to robust rate-remapping is also strong enough in some cases to move the fields independently, depending on which type of inputs dominate the synaptic input vector of a given cell. The fact that this effect occurs predominantly in CA1, which lacks the potential stabilizing effects of reciprocal excitatory connections present in CA3, tends to support such a view[127]. A second possible challenge is the fact that place fields can be expressed in CA1 under conditions in which the medial entorhinal cortex (MEC) is completely lesioned[128]. This suggests that localized firing may itself be generated from alternative inputs, such as from weakly spatially modulated neurons in the lateral entorhinal cortex (LEC)[129], which may provide hippocampal cells with path-integration-independent sensory inputs necessary for efficient rate coding[130]. However, even under conditions in which MEC inactivation does not impair hippocampal place selectivity, the intervention causes instant remapping[131,132], suggesting that MEC is obligatory for activating the correct place map. This does not preclude, of course, that place maps are also stored in the CA3 network (for example, the 'charts' of Samsonovich and McNaughton[90]), or that, in the absence of a strong MEC input, CA3 attractor dynamics may result in the recall of some previously constructed chart in the novel context.

Moving from hippocampus to entorhinal cortex

Until the 1990s, for primarily technical reasons, most recording studies had been confined to CA1 of the dorsal hippocampus, in spite of the fact that hippocampal subfields may have distinct computational functions. David Marr had, in the early 1970s, already pointed to the unique properties of area CA3 as a recurrent network capable of auto-association, pattern formation, and pattern completion[133]. His work was followed by theoretical investigations pointing to the possible role of the dentate gyrus in pattern-separation processes needed to counteract memory interference at subsequent stages of the hippocampal circuit[134–136]. An additional, striking property that was discovered to differentiate between hippocampal subfields was coding sparsity. Contrary to some expectations, in the successive transformations from CA3 to CA1 to subiculum, mean firing rates increased, and coding became less sparse and less spatially selective[137,138]. This observation led Barnes *et al.* to conclude that "discrete spatial representations are constructed within early stages of the process, for some purpose intrinsic to the hippocampus itself, possibly that of rapid information storage" and that "the information leaving the hippocampus through the subiculum seems to consist of much more highly distributed representations, constructed perhaps through the convergence and disjunction of a number of unrelated hippocampal place cells"[137]. For a long time, however, these ideas did not fully catch the attention of the place cell community, which, with few exceptions, retained its focus on the readily accessible CA1 area.

In a similar manner, until the 1990s, there was minimal focus on computational operations outside the hippocampus and computations underlying place-field formation were at risk of being erroneously attributed to the hippocampus itself. The focus on a hippocampal origin of the place cell signal was further influenced by the observations of a relatively small set of tetrode studies in the entorhinal cortex, the major cortical input to the hippocampus. These studies showed that entorhinal cells were spatially modulated but that their firing fields were broad and dispersed, with little spatial selectivity in standard laboratory environments, and the fields seemed not to remap between environments[22,137,139]. This, together with the observation that CA1 place fields persisted following large lesions of the dentate gyrus[140], pointed to the remaining associative networks of CA3 as one possible origin for the formation or learning of the sharply localized place signals seen in CA1. The validity of this interpretation was questioned, however, by the fact that partial inactivation of CA3 cells, following inhibition of septal inputs, failed to remove spatial firing in CA1[141].

Given the uncertainty about how CA3 contributed to the CA1 place signal, Brun and colleagues[142] decided to record place cells in CA1 after the CA3 input to these cells had been entirely removed by excitotoxins or by knife cuts that completely separated CA1 from CA3 as well as from dentate gyrus and subcortical afferent regions. Retrograde tracer injections in CA1 verified that no input was spared. Confirming the interpretation of the septal-inactivation work[141], the study found, in 2002, that CA1 place cells do not require input from CA3 to maintain reasonably selective spatial firing. This suggested either that place fields were generated within the limited circuitry of the CA1 itself or that place cells in CA1 received spatial input from the entorhinal cortex via temporoammonic projections that survived the CA3–CA1 transection. These observations were made only a few years after theoretical studies[3,21,90,143] proposed that the path integrator might located outside the hippocampus—in the

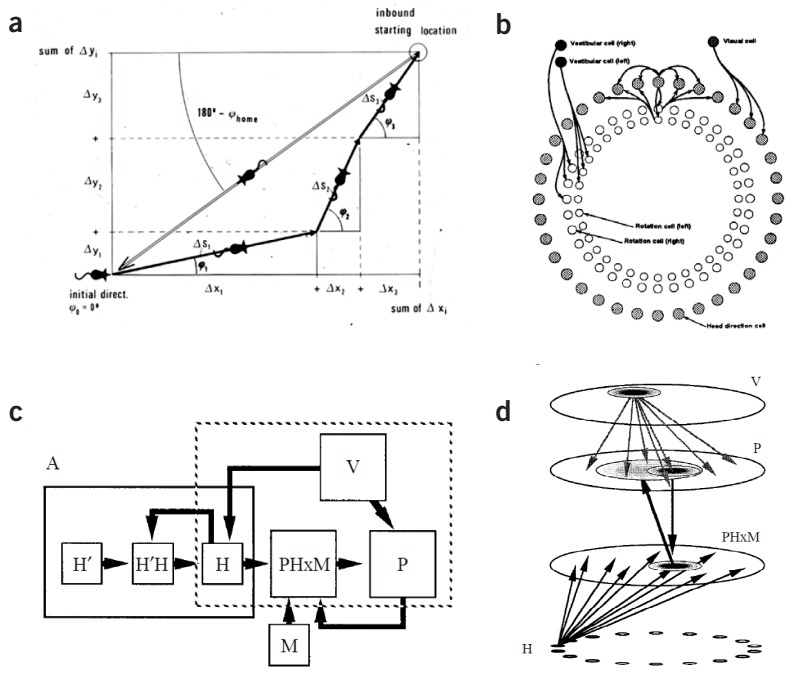


Figure 5 Path integration. (**a**) Illustration of the Mittlestaedt & Mittlestaedt 1980 experiment[12]. This experiment showed that rodents can perform angular and linear path integration. A female mouse returns directly to her nest after finding a lost pup in total darkness but makes a heading error if she is rotated below vestibular threshold before starting the inbound journey. (**b**) The Skaggs *et al.* continuous-attractor model from 1995 proposed to explain how head direction cells arise through integration of head angular velocity signals from the vestibular system[18,232]. Updates in the head direction (attractor) layer were performed by a hidden layer of cells conjunctive for head angular velocity and starting head direction, whose return projections to the head direction layer are offset according to the sign of rotation. Such conjunctive cells have been found in several regions of the brain. (**c**,**d**) The continuous-attractor model for path integration in two dimensions, as proposed by McNaughton *et al.* in 1996 (ref. 19) and simulated by Samsonovich and McNaughton in 1997 (ref. 90). H′, head angular velocity; H′H, conjunctive cells; H, head direction; P, place cells; M, speed cells; PH×M, cells conjunctive for place and head direction and modulated by speed; V, external sensory inputs that were assumed to associatively bind to both H cells and P cells to enable correction of drift error in the path integrator and to enable resetting of the integrator upon entry to a familiar environment. Panel **a** reproduced with permission from ref. 91, Nature Publishing Group. Panel **b** reproduced with permission from ref. 232, MIT Press. Panels **c** and **d** reproduced with permission from ref. 90, "Path integration and cognitive mapping in a continuous attractor neural network model," A. Samsonovich & B.L. McNaughton, 1997, in *Journal of Neuroscience*, Vol. 17, page 5900–5920.

subiculum, the entorhinal cortex, or both—because correlations between firing fields in these regions appeared to be invariant across contexts[22,23], as might be expected for a path-integration-based representation. At this time it was clear that the entorhinal cortex, the main cortical input to the hippocampus, was worth a revisit.

An important additional inspiration for the renewed interest in entorhinal cortex was Menno Witter's extensive review of entorhinal–hippocampal systems[144]. Witter pointed out that dorsal and ventral regions of the hippocampus receive inputs from and project back to different regions of the entorhinal cortex, in a topographical manner, with increasingly dorsal hippocampal regions mapping onto areas that were increasingly closer to the rhinal sulcus, or increasingly more dorsal within the MEC. In 1990, based on his review and after direct consultation with Witter, two of us (M.-B.M. and E.I.M.) realized that in earlier MEC recordings for which histology was available[22,139], cells had been recorded quite far outside the area of MEC that receives most visual–tactile information and projects most extensively to the dorsal hippocampus, where the most sharply tuned place cells of the hippocampus are located[80,81]. This led us, eventually, after the turn of the millennium, to target tetrodes to the dorsal MEC, the origin of the majority of inputs to the dorsal hippocampus[8,144], a region of MEC so far not touched by electrodes *in vivo*.

Grid cells: a metric for space?

Recordings in dorsal MEC soon showed that cells in this region have sharply defined firing fields, much like those in CA1 of the dorsal hippocampus, except that each cell had multiple firing fields, distributed all over the environment[8]. These findings, reported in 2004, pointed to the MEC as a key element of a circuit for space, but the nature of the entorhinal representation remained elusive.

A striking characteristic of many spatially modulated MEC cells was that the distribution of the multiple firing fields of each cell was more regular than expected by chance[8]. When the data from MEC were presented at the 2004 Society for Neuroscience meeting, they created considerable excitement. Among those who were most excited was Bill Skaggs, who thought he saw hexagonal symmetry, inspiring the Mosers and their students, Hafting, Fyhn, and Molden, to increase the size of the recording arena and visualize the firing pattern once and for all. Using a newly constructed 2-m-wide circular recording cylinder, these authors found, in a substantial fraction of MEC superficial-layer cells, that the firing fields of individual cells created a grid-like periodic hexagonal pattern tiling the entire space available to the animal[9] (**Fig. 7a**). These cells were designated as grid cells. For each cell, the grid could be assigned a phase (the *x*,*y* locations of the grid vertices), a wavelength or spacing (the distance between the vertices), and an orientation (how much the axes through the vertices were tilted compared to an external reference line). In addition, the peak firing rates varied between fields[9,145]. The spatial periodicity of the pattern was so striking that the authors were concerned, initially, that it was some sort of artifact. However, the grid pattern was soon found by other labs too[129,146].

One of the most striking aspects of the grid cell finding was that the spatial periodicity was maintained despite constant changes in the animal's running speed and running direction. The cells fired at the same vertices regardless of how much time and space the rat had traveled between each crossing, implying that grid cells had continuous access to information about distance and direction moved. The persistence of grid fields[9] and place fields[70] when rats run in darkness is consistent with the primary role that such self-motion information might have in determining firing locations, as is the fact that grid patterns unfold immediately in new environments[9] and are expressed with similar phase relationships between cell pairs in all environments tested[145]. It should be added, for the sake of balance, that stable

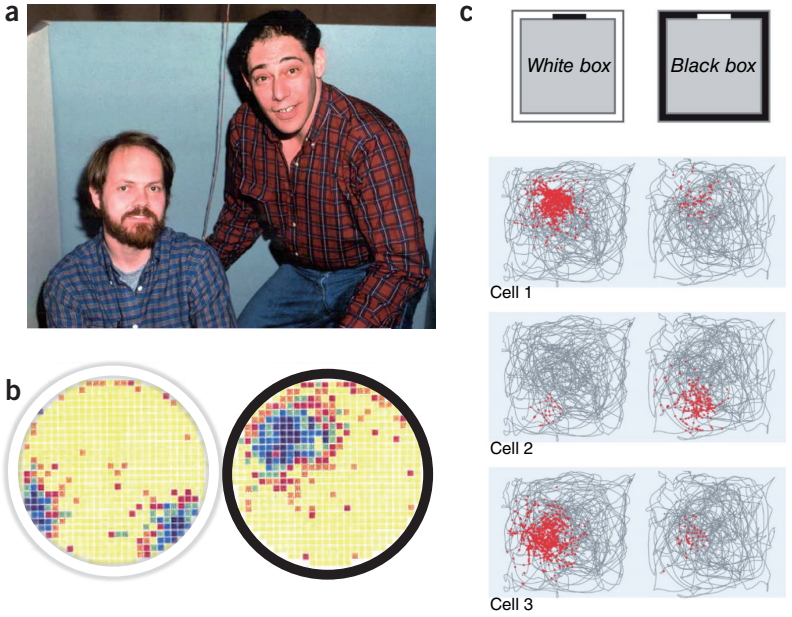


Figure 6 Remapping. (**a**) John Kubie and Robert Muller from SUNY Downstate Medical Center, NY. Picture courtesy of John Kubie. (**b**) Global remapping apparently induced by changing only the color of the recording environment[100]. Rate maps are shown for the same place cell recorded in a white cylinder (left) and a black cylinder (right). Firing rate is color-coded from yellow (no firing) to dark blue or black (high rate). The cell fires in different regions of the cylinder (some cells are active in only one cylinder) despite changing only the color of the box. We note that the authors later confirmed, anecdotally, that they had pretrained the animals in the white and black cylinder in two different rooms, which would have allowed differences in path-integrator coordinates to control the global remapping, as later shown by Colgin *et al.*[111]. (**c**) Rate remapping induced by changing the color of the recording environment while keeping its location constant[106]. The rat's trajectory in a white box and a black box is shown for three cells, with spikes superimposed as red dots. Note that changing only the color of the box causes substantial change in the distribution of firing rates across cells, but firing locations are retained. Rate maps in **a** adapted with permission from ref. 100, Wiley. Panel **c** adapted with permission from ref. 265, Elsevier.

grid fields have not yet been identified in darkness in mice[147,148]. The reason for the possible species difference is not known. Associations between path-integration coordinates and stationary cues may be weaker in mice[149], or grid fields of mice may simply be harder to visualize at times of increased jitter, given their smaller field size and shorter grid spacing compared to rats[150].

Based on the possible role of self-motion information in the formation of grid patterns, the three of us suggested, in 2006, that grid cells are part of an intrinsic path-integration-based metric for space[91]. A similar proposal was made the same year by a different group of investigators[151]. Both concepts bore similarities to the mechanism proposed a decade earlier from studies of place cells[19,90]. In fact, by implementing their attractor map model for path integration on a torus, Samsonovich and McNaughton[90] indirectly predicted periodic place fields, although, at the time, the idea seemed to them too preposterous to publish, and an attempt to discover such periodicity in CA1 by running rats down a long hallway concluded that "place field distributions can best be described by a random selection with replacement"[152]. A decade later, with the new data from the entorhinal cortex, it was clear that grid cells may supply the brain's spatial map with a coordinate system not available from place cells in the hippocampus, given the apparently random allocation of place fields to position[153] and the related extreme remapping across environments.

It soon turned out that if grid cells supply a metric, this metric is not always constant over time or locations. Experiments showed that when environments were stretched or rescaled, the spacing of the grid increased in the extended direction[146,154], in concert with either scaling or remapping in hippocampal place cells[155]. However, these distortions of the grid pattern were recorded when the environment was changed after the animal was already familiar with it, suggesting that grid maps might be formed by path integration but linked to external cues in such a way that the latter can override the path-integration dynamics[90]. Yet under some conditions, grid cells appear to be fragmented or distorted even after extended training in a constantly shaped environment. When rats are tested in environments with discrete compartments[156] or irregular geometric shapes[157], the strict periodicity of the grid pattern is often gone. In particular, it has been shown that walls exert strong local influences on the grid pattern[157,158], causing distortions and rotations that can be described effectively as a shearing process[158]. The common presence of fragmented and distorted grids has raised questions about whether grid cells are useful as a source of metric information[157]. Countering these doubts, theoretical analyses have shown that precise symmetry may not be necessary for accurate population-based decoding of position, distance, and direction if the grid cells are all distorted in the same way[159]. Direct behavioral evidence is needed, however, to establish how well spatial metrics can be decoded from distorted grid patterns.

Network properties of grid cells

Grid cells differ from place cells in more than one way. Not only do they have periodic firing fields but the relationship between the firing fields of different cells also follows a different rule. Whereas place cells often remap completely between environments and multiple fields can appear in large environments, with no more overlap in the subset of active cells than expected by chance[106,153,160–162], the ensemble activity of grid cells is normally maintained coherently from one environment to the next, without changing phase or orientation relationships between cells[145,163], much like in early recordings from MEC cells before grid cells were discovered[22]. The coherence of the grid map is particularly strong within ensembles, or modules, of similarly scaled grid cells[154]. A similar degree of coherence is present among head direction cells[6,7,77,78,164], as well as in the more recently discovered populations of entorhinal border cells and speed cells[165,166]. The coherence of grid cells and head direction cells is state-independent and persists during sleep[167–169]. Collectively, these findings point to a fundamental difference between hippocampal and entorhinal spatial maps: hippocampal circuits are high-dimensional and capable of storing a very large number of patterns, while MEC maps are low-dimensional and rigid, expressing the same intrinsic structure in all behavioral contexts, as would be expected for a path-integration-based map that keeps metric properties constant across contexts and environments.

It was clear from the outset that grid cells come in different varieties—with different

phases, wavelengths, orientations, and field amplitudes—and that the network of grid cells is anatomically organized according to some but not all of these variables[8,9]. While the phase of the grid pattern appeared to be distributed randomly among cells on the same tetrode, the scale of the grid showed a striking increase from dorsal to ventral recording locations in the MEC (**Fig. 7b**). In both respects, the organization of grid cells was reminiscent of that of place cells, which also appear to have random spatial relationships[160,170,171] but show an increase in scale from dorsal to ventral[80,81]. In the hippocampus, the scale increase is strongly coupled with decreasing gain of self-motion parameters[84,86]. A similar gain-change may underlie the scale change in MEC, consistent with the hypothesis that the overall system parameters are dominated by path-integration mechanisms.

One question that was not settled by the earliest grid cell recordings was whether the scale gradients were smooth and gradual or instead consisted of multiple discrete maps with distinguishable scale and self-motion gain, the latter being a necessary prediction of attractor-map-based models[91,172]. In 2007, Barry and colleagues showed, with a small cell sample, that values of grid spacing were not evenly distributed[146]. In 2012, Stensola and colleagues were able to record activity from up to 180 grid cells in the same animal: enough to determine once and for all whether grid cells clustered in groups with similar properties[154]. Stensola *et al.* found that grid cells were organized in at least four modules, each with their own scale, orientation, and asymmetric distortions (**Fig. 7c**). The scale change across successive grid modules could be described as a geometric progression with a constant scale factor[154], confirming the prior predictions[91,172], as well as theoretical analyses pointing to nested and modular organizations as the most efficient code for representing space at the highest-possible resolution with the lowest-possible cell number[173,174].

The discovery of grid cells cast new light on the mechanisms underlying formation of place cells, the very question that motivated the search for spatially modulated cells in the entorhinal cortex. The periodicity of the firing pattern and the variability of the grid scale suggested early on that place cells may emerge by a Fourier-like linear summation of output from grid cells with similar phase throughout the environment over a range of spatial scales[91,175]. This summation mechanism might be facilitated further by coordinated gamma-frequency oscillations in MEC and CA1 cells[176]. Alternatively, and more in line with the sensory-integration ideas of the 1980s, place fields might be generated from any weak spatial input, so long as the hippocampal circuit contains mechanisms for amplifying a subset of these inputs, either through Hebbian plasticity or through local recurrent networks[177–180]. The merits of these two classes of models remain to be determined. Experimental studies have shown that MEC grid cells are not necessary for the emergence of spatially tuned firing in place cells. Place fields have been reported to persist when the spatially periodic firing pattern of MEC grid cells is compromised by inactivation of septal inputs[181,182], and in young animals, place cells acquire stable firing fields before sharp periodic firing patterns emerge in grid cells[183,184]. Inactivation or damage of the MEC is not sufficient to disrupt place cell firing in the hippocampus[128,131,132,185]. However, neither of these observations rules out grid cells as a key determinant of spatially selective firing in the hippocampus. The hippocampus receives input from multiple spatially tuned entorhinal cell types, including not only grid cells but also border cells and spatially modulated cells with nonperiodic firing patterns[186], as well as weakly place-tuned cells in the LEC[129]. Place fields may be formed from any of these inputs, by more than a single mechanism. Even pure rate changes among the MEC inputs are sufficient to completely alter the activity distribution among place cells in the hippocampus[185]. The mechanism for grid cell to place cell or place cell to grid cell transformation may have many faces, and understanding it may require that circuitry is disentangled at a higher level of detail, possibly in terms of inputs and outputs of individual cells.

A zoo of cell types

Grid cells are abundant, especially in the superficial layers of the MEC, but not all cells are grid cells. As early as 2006, it was clear that in layers III–VI of the rat MEC, a number of cells respond to head direction[187] (**Fig. 7d**), very much like the head direction cells reported in the neighboring presubiculum and parasubiculum years before[5–7,188]. The directional tuning curves of many entorhinal head direction cells were found to be broader than in presubiculum and parasubiculum, and many head direction cells responded conjunctively to location, expressing grid-like firing fields but discharging within each grid field only when the rat's face pointed in a certain direction[187]. Head direction cells intermingled with grid cells and conjunctive grid × head direction cells (**Fig. 7e**) throughout MEC layers III–VI, as well as in presubiculum and parasubiculum[189], pointing to a computational mechanism for imposing the angular component of path integration on grid cells[19,91].

Shortly after head direction cells were observed in recordings from the MEC, another cell type appeared on the entorhinal stage. These cells, named border cells, fired exclusively along geometric borders of the local environment: along one or sometimes several walls of the recording enclosure or along the edges of a platform[165,190] (**Fig. 7f**). Border cells were distinct from grid cells—a border cell could never be transformed to a grid cell or vice versa—but there was overlap between border cells and head direction cells, i.e., some (conjunctive) border cells fired within their border fields only when the animal was running in one direction[165]. Border cells intermingled with grid cells and head direction cells, particularly in layers II and III of MEC[165], suggesting that the three types of cells interact. However, while grid cells and head direction cells seemed to be confined to parahippocampal—and not hippocampal—regions, cells with border-like firing fields were also observed in the hippocampus[191] and the subiculum[192,193], raising the possibility that firing patterns of entorhinal border cells are inherited by at least subsets of neurons in the hippocampus and subiculum[93,194], or vice versa.

Border cells are sparser than grid cells and head direction modulated cells, and they may comprise less than 10% of the local principal cell population[165], but this does not negate a significant role in shaping hippocampal–entorhinal representations. The discovery of border-like properties in several regions of the hippocampal formation confirmed, to some extent, predictions from computational models dating back to the observation that the location and shape of place fields are determined by local boundaries of the recording environment[93]. Based on this observation, O'Keefe, Burgess, and colleagues proposed a model in which place fields are formed by summation of tuning curves from upstream 'boundary vector cells', cells with firing fields tuned to the animal's distance from a particular wall or boundary in the environment[93,192,194]. Boundary-vector-like cells, with distance-dependent tuning curves, were reported in the subiculum[193], but, given the unidirectional wiring of the hippocampal circuit, these cells are unlikely to provide major input to hippocampal place cells. Such inputs might instead come from border cells in the MEC. On the other hand, border cells in MEC lack distance tuning, firing only along the bor-

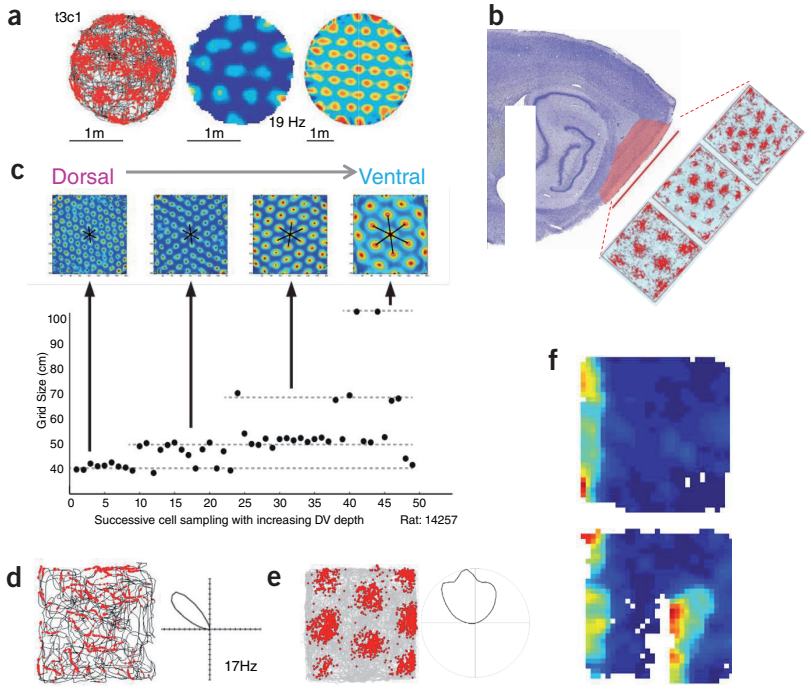


Figure 7 Grid cells and other functional cell types of the MEC. (**a**) Firing fields of one of the first grid cells reported in 2005 (ref. 9). Left: trajectory of the rat (black) with superimposed spike locations (red). Middle: color-coded rate map with peak rate indicated (red, peak rate; dark blue, no firing). Right: spatial autocorrelogram, color-coded from blue ($r = -1$) through green ($r = 0$) to red ($r = 1$). (**b**) Sagittal section of the rat brain showing the hippocampus and the MEC (red) and grid cells of different scales recorded at three locations on the dorsoventral axis (trajectories with spike locations as in **a**). Note the expansion of grid scale from dorsal to ventral MEC. (**c**) Grid cell modules[154]. Top: autocorrelation plots showing grid patterns at successive positions along the dorsoventral axis of MEC. Bottom: grid size, defined as the distance between grid vertices, as a function of position along the dorsoventral MEC axis (positions rank-ordered). Note that the increase in grid size is not linear but discretized, following a geometric order with a factor of approximately $\sqrt{2}$. Mean grid size for each module is indicated by stippled lines. Such modularization is an essential prediction of the attractor map theory if it is to account for variable spatial scaling[91]. (**d**) Head direction cell in layer V of MEC. (**e**) Conjunctive grid × head direction cell in layer III of MEC. (**f**) Border cell[165]. Color-coded rate maps showing a cell with selective firing along one of the walls of the recording environment. Top: open environment. Bottom: rate map following the insertion of a wall. Note that the border cell responds to the same side of the wall insert as the main wall in the environment. Panel **a** reproduced with permission from ref. 9, Nature Publishing Group. Panel **b** adapted with permission from ref. 91, Nature Publishing Group. Panel **c** adapted with permission from ref. 154, Nature Publishing Group. Panels **d** and **e** adapted with permission from ref. 187, AAAS. Panel **f** adapted with permission from ref. 165, AAAS.

ders and not away from them. If border cells provide input to place cells, their influence might be limited to cells with firing fields in the periphery of the recording enclosure, near boundaries and not in open spaces. There is some indirect evidence for this possibility as, in juvenile rats, place cells with fields in the center of an open recording environment mature at the same slow rate as grid cells[195], which acquire adult-like hexagonal symmetry only late in juvenile development[183,184]. Place cells near the borders of the recording box appear at an earlier age, similarly to entorhinal border cells[196]. Regardless of whether border cells fulfill criteria for boundary vector cells or not, the existence of border cells, as well as the strong asymmetries in grid patterns caused by environmental boundaries[157,158], point to a significant role for boundaries in defining the location of firing in place cells and grid cells, consistent with behavioral studies identifying geometry of the environment as a determinant of the animal's perception of self-location[13,197,198]. However, these observations are not at variance with a path-integration-based account of spatial firing of grid cells. Boundaries may serve as references for path-integration-based position estimates, with resetting of the path integrator and subsequent reduction of error taking place regularly near major boundaries or landmarks[19–21,90]. The increased variability of grid field locations in open spaces compared to locations near the walls[199], as well as the instability of place fields in open spaces when spatially stable information is available only from border cells[195], speak in favor of a reference function for environmental boundaries, where grid and place representations are reset and corrected from drift each time the animal encounters a salient boundary.

With the identification of head direction cells and border cells, it became clear that grid cells have local access to directional information, needed for the angular component of path integration, as well as to information about the geometry of the environment needed to prevent drift in the path-integrator coordinates. Head velocity signals upstream of head direction cells, in the lateral mammillary nuclei[200] and further upstream in the dorsal tegmental nuclei[201,202], might enable head direction cells to infer direction at the timescale of behavior. However, if grid cells express path integration, they must also have access to information about moment-to-moment changes in the animal's speed. Such information was known early on to be present in the hippocampus, where both place cells and fast-spiking interneurons exhibit speed tuning[30,86,203]. Speed-responsive cells have similarly been observed in subcortical areas directly or indirectly connected with hippocampal and parahippocampal regions[204–207]. These cells might feed into the brain's path-integration system. Speed tuning of hippocampal theta rhythm amplitude is sufficient to enable accurate reconstruction of distance traveled[208], and distance traveled might be decoded by integrating the net discharge rate of a population of hippocampal cells or afferents of the hippocampus.

The observation of speed coding in the hippocampus and subcortical areas motivated the search for speed information locally within the MEC circuit. By 2006 it was observed that some information about speed is present in a subset of grid cells, especially in layer III and deeper[187], but the correlations between firing rate and speed in these cells were weak and would require decoding from large cell numbers to yield a reliable momentary speed signal[166]. We now know that the entorhinal cortex has a distinct population of cells whose firing rates increase linearly with speed[166,209]. In the large majority of speed-tuned MEC cells[166], firing rates increase linearly as a function of speed, up to 30–40 cm per s in rats. A small but significant number of cells have negative speed–rate relationships[166]. As in the hippocampus, many of these are fast-spiking cells[210]. The rates of these cells are tuned so

strongly to running speed that speed can be decoded with extreme accuracy from just half a dozen cells[166]. Tuning profiles (slope and *y*-intercept of the speed–rate relationship) vary between speed cells but remain constant across environments and persist in the absence of visual cues, pointing to speed cells as yet another component of a low-dimensional path-integration-based position map in the MEC[166]. In CA1, the gain of speed tuning varies systematically along the septo-temporal axis in register with the change in spatial scale[86]. This has yet to be confirmed in MEC, but if verified it would strongly support the idea that speed cells convey the necessary information to set the grid scale.

Taken together, these observations point to a network of entorhinal and hippocampal neurons in which position, direction, and distance are encoded with sufficient accuracy to enable dynamic representation of the animal's location in an empty enclosure. However, most real-world environments differ from experimental settings, in that the available space is cluttered with objects. Salient objects may serve as references for navigation, but little is known about whether and how objects are included in the representation of self-position in the MEC. It has been shown that a subset of neurons in the LEC respond specifically at the locations of discrete objects in the recording enclosure[211,212]. These neurons increase firing whenever the animal encounters an object at a certain location, regardless of the exact identity of the object. In a subset of these object cells, firing even persists for minutes, days, or weeks after the object is removed[212]. Whether and how these cells contribute to representation of the animal's own location has remained elusive. Theoretical models from the 1990s postulated the existence of cells with place fields, defined by the animal's vectorial relationship to salient landmarks in allocentric coordinates[213], and such cells are indeed found in small numbers in the hippocampus[214]. These cells encode direction and distance from one or a small number of discrete objects placed at different locations in the recording arena. Now new data suggest that a class of MEC cells has more general vectorial properties. These 'object vector cells' have firing fields defined by distance and direction from an object, regardless of the object's location in the environment and regardless of what the object is[215]. Thus, one main difference between object vector cells in MEC and in CA1 appears to lie in their object specificity. Perhaps, like rate remapping of hippocampal place cells, the coordinate information in CA1 is inherited from MEC, whereas the identity information is added after the fact, possibly from LEC[129,130,211,212]. Like rate remapping in place cells[216], at least some of the CA1 object vector cells appear to require extended experience[214].

Finally, investigators have identified a population of hippocampal cells with activity defined by the animal's egocentric orientation to a goal location. Sarel *et al.*[217] recorded from the CA1 region of flying bats, which have hippocampal–parahippocampal spatial representations similar to that of rodents[218–220]. The investigators identified a set of cells that responded as a function of the animal's orientation toward a salient goal positioned centrally in the environment. Although the preferred orientation of the cells spanned the full 360° range relative to the direction to the goal, a large proportion of the cells in this category fired when the animal was heading directly toward the goal, ramping up their firing as the bat approached the goal. A little more than half of the cells were also place cells, but a substantial fraction did not have any significant tuning to place. Cells with essentially the same characteristics were recently reported in posterior parietal cortex[17]. Goal-vector cells are reminiscent of cells reported in rats in earlier hippocampal studies, in which neural firing increased in the proximity of a goal[73,221–225], and the finding of goal-orientation cells in both parietal cortex and hippocampus begs the question of which region is 'copying' which. Future research may determine whether similar cells are also present in the MEC circuit and whether they remap between goals and environments, like place cells, or maintain intrinsic spatial and directional relationships, like all medial entorhinal functional cell types characterized so far.

The multitude of functionally specialized cell types in the entorhinal–hippocampal space circuit is striking; however, equally striking is that many cells still express more than one type of information, particularly in the intermediate and deep layers of MEC, where many grid cells fire conjunctively for position and head direction, or position and speed, and many border cells are direction-selective[165,166,187,226]. Conjunctive cells are recognized as essential ingredients of the 'hidden layer' for almost any type of coordinate transformation or conditional association network[18,227–229]. A challenge for future work will be to determine how this variety and mixture of differently tuned cell types enable a dynamic representation of self-position that can be read out to guide navigation and memory for a wide variety of environments.

The role of theory: mechanisms of place cells, head direction cells, and grid cells

The abundance of functionally dedicated cell types in the entorhinal–hippocampal system has prompted investigators to look for the neural mechanisms that enable their characteristic firing patterns. Mechanisms have been sought in the properties of single cells as well as in neural networks. While details remain elusive, the preceding sections of this review have already emphasized how circumstantial evidence points to path-integration-based attractor-network properties as a key contributor to pattern formation in the entorhinal–hippocampal space system.

Attractor networks have provided starting points for models of localized firing since the earliest studies of hippocampal function. In 1949, Hebb proposed that activity may self-sustain in networks of recurrently connected neurons[230]. In 1977, Amari took a giant step by showing that localized firing can be maintained in networks of neurons arranged conceptually on a ring with Mexican-hat connectivity[231]. In such architecture, each neuron has strong excitatory connections to its nearest neighbors, with excitation decreasing with distance along the ring, in contrast to inhibition, which is maintained at longer distances. Almost 20 years later, Skaggs and McNaughton and colleagues[232]; Zhang[233]; and Redish, Touretzky, and colleagues[234] showed, independently, how the concept of a ring attractor with local (Gaussian) connectivity and global recurrent inhibition could be used to explain the emergence of directionally specific firing in head direction cells (**Fig. 5b**). The connectivity created a self-maintained activity bump, which could be induced to move around the ring in accordance with external angular velocity signals that were transmitted through a hidden layer of conjunctive head direction × angular velocity cells[18]. The model explained a number of features of head direction cells, including the persistence of directional phase relationships across conditions and environments. Today, more than 20 years after its proposal, the key concepts of the ring-attractor model for head direction cells remain unchallenged, which is remarkable for theoretical models in systems neuroscience, and no competing models have surfaced. In mammals, the reciprocally connected network of the dorsal tegmental nucleus and lateral mammillary area has been proposed as a location for the ring attractor[235], and in *Drosophila*, the concept of a ring attractor for directional tuning has received its first experimental support in studies of central body neurons, where a circular anatomical arrangement has been shown to

Box 1 Questions for the future

We have listed some outstanding problems in entorhinal–hippocampal space circuits that we believe can be addressed with state-of-the-art systems neuroscience tools.

1. Path-integration networks and mechanisms of grid cells and head direction cells
The performance of attractor network models for space relies on a unique and testable connectivity between functionally similar cells. With state-of-the-art tools for neural imaging, genetic tagging, and structural analysis, it may soon be possible to examine directly, in large MEC populations, the probability of connections between functionally identified neurons with various degrees of feature similarity and dissimilarity. On a longer time scale, one may hope for a direct visualization, with *in vivo* microscopy, of activity flow between connected mammalian neurons in a way that matches the animal's movement in space (similar to refs. 236,237 in flies).

2. Development of spatial network architectures
How is the specificity of the hippocampal–entorhinal spatial neural network architectures achieved during development of the nervous system? Excitatory neurons from the same radial glial progenitor are known to have stronger interconnections than other cells[266,267]. Might such connectivity between clonally related cells underlie a possible preferential coupling between MEC cells with similar spatial or directional tuning, in the same way that cells from the same clone exhibit similarities in orientation preferences (and possibly preferential coupling) in the visual cortex[268,269]? Does the young MEC have a topographically arranged teaching layer, with connections between clonally related cells, that during early postnatal development gives way to the largely nontopographical[9,270] grid cell network of the adult MEC (Fig. 8 of ref. 91)? Tools have been developed for targeted analysis of the functional identity and connectivity of discrete developmental cell populations, allowing these questions to be resolved in the near future[271].

3. Including the entire entorhinal–hippocampal circuit
A key objective for a more complete understanding of entorhinal–hippocampal function will be to determine how cell types with different functional correlates map onto the variety of morphological or neurochemical cell types and their unique connectivity patterns. Recent data suggest that, in layer II of MEC, both stellate and pyramidal cells can be grid cells, although stellate cells may comprise the majority of them[256,257,272–275]. If so, are grid patterns created independently in these two cell classes, or does one of them inherit the grid from the other?

4. Read-out
Position can be decoded from grid cells and place cells, with greater accuracy in grid cells than place cells if the population is multimodular and scaled in particular ways[159,173,174,276]. Whether neural circuits decode information in the same way remains to be determined, however. Do neurons have access to grid cells with different phase relationships or different spacing; do they integrate information from grid cells with information from border cells or head direction cells? If so, where are these neurons and how do they communicate with neocortical regions involved in strategy formation and decision-making? Most research on the mechanisms of spatial coding in hippocampus has focused on the nature of the inputs that contribute to it, and less is known about the impact of hippocampal output on coding dynamics in the widespread regions of neocortex and other areas to which the hippocampal formation projects. The impact of outputs from the entorhinal–hippocampal circuit will perhaps constitute a new frontier in the study of this system.

5. Moving toward naturalistic environments
Natural environments are large, three-dimensional, compartmentalized, nested, and full of objects. Ultimately, studies of the hippocampal–entorhinal circuit should explore how cells map environments of shapes, sizes, and content more comparable to the animal's natural habitat[277]. Are grid cells, head direction cells, and place cells used only for local mapping, in the range of a few meters, or is the entorhinal–hippocampal network used also for extended spaces, and if so, how? Is there a single continuous map, or are there different maps for different local spaces, as proposed by theoretical studies[278], as well as observations in compartmentalized laboratory environments[156]? If the latter is true, how are the map fragments connected? And how is space coded in large and three-dimensional environments[277]? In flying bats, place cells have spherical firing fields[279] and head direction cells are tuned to all three axes of orientation[220]. Whether such volumetric coding extends to terrestrial animals remains unsettled, although experimental data suggest that, in rats, head direction is encoded not only by classical azimuth-sensitive head direction cells but also by cells in the lateral mammillary bodies that respond to head pitch[200]. Observations in rats also suggest that the tilt of a surface is factored into hippocampal and entorhinal representations of space[280,281].

6. Representation of time
Understanding space and memory requires understanding time. Direct representation of the passage of time was not observed in hippocampal neurons until the Buzsáki and Eichenbaum groups showed that, when animals run for a known interval at a steady location, in a running wheel[282] or on a treadmill[283], hippocampal neurons fire successively at distinct times during the interval, following the same order on each trial. Cells with similar properties are present in the MEC[284]. Most of these 'time cells' have discrete place or grid fields in standard spatial foraging tasks. Different assemblies and sequences of hippocampal time cells are active in different task configurations[283], suggesting that hippocampal ensembles encode temporally organized information much the same way they represent space. The observation of time cells is a provocative finding that may share properties with mechanisms underlying path-integration-based representation of location, but the temporally confined firing fields of time cells do not disappear when time and distance are decoupled by restraining

(continued)

Box 1 (continued)

the animal[285] or changing the speed of the treadmill[286], suggesting that sequences do not exclusively reflect the number of steps at the task location. Certainly the relationship between representations of space and time and the role of time cells in perception and recall of time require further study. While time cells have firing fields in the order of a few seconds, and assemblies of time cells can represent events at the scale of tens of seconds, encoding of longer temporal distances may require different mechanisms. One may speculate that the spontaneous drift over hours and days in the firing properties of place cells in CA2 and (to a lesser extent) CA1 (refs. 287–289), as well as cell populations in LEC[290], may possess the power to encode temporally distant events as distinguishable memories.

7. Beyond physical space

Do grid cells and other spatially modulated cells encode information beyond physical space, as suggested by O'Keefe and Nadel[65]? Evidence for such an extension of functions was reported recently in a task in which rats press a lever to alter the frequency of a sound on a continuous scale; in this experiment, hippocampal and entorhinal cells display frequency fields resembling place fields during navigation of physical space[291]. Further functional expansion might be expected in primates. Indeed, in monkeys, hippocampal and entorhinal cells fire in patterns defined not by the animal's location in space but by where it moves its eyes on a visual scene[255,292,293]. This observation raises the possibility that place and grid cells create a map of visual space using eye movement signals instead of locomotor information to support coordinate transformation, without having to change any other computational elements of the circuit. In humans[294,295], grid cells may take on functions in conceptual mapping[296]. The possible adoption of grid cells as a metric for navigating abstract spaces would be consistent with the idea that hippocampal circuits first evolved for representation of space and later acquired the capacity for imaginary navigation[49,65,297,298]. This expansion of functions would be reminiscent of the way cortices originally involved in object recognition formed the basis for a visual word form area during the evolution of written language processing in the human cortex[299].

underlie firing in neurons that represent orientation relative to landmarks[236,237].

Only a year after the introduction of velocity-driven ring attractors to models of head direction cells, it was acknowledged that a similar integration mechanism might apply for position mapping in two dimensions, as expressed in hippocampal place cells[19,90,233,238,239] (**Fig. 5c,d**). In the position version of the model, neurons were arranged conceptually according to their location of firing in two-dimensional space. A matrix of recurrent connections was generated, in which excitation decreased with the distance between neurons on the sheet. In combination with global inhibition, self-excitation between similarly tuned cells maintained localized firing. A path-integration mechanism moved the activity bump across the network in accordance with the animal's position in the environment, using conjunctive head direction × place cells, in the same way that angular velocity inputs moved the bump in the ring attractor for head direction cells. The model was proposed to apply for any neural architecture of the hippocampal system, but with the knowledge that existed in the 1990s, the implementation was focused on area CA3 of the hippocampus. This explained a number of properties of place cells but faced one major challenge: the subset of active hippocampal neurons remaps across environments and circumstances[71,100–102]. For position to be computed in place cells, some sort of independent architecture for each environment would then be required. This is computationally possible[90,240] but nonetheless raises the question of whether a single network matrix, expressed in all environments, would not be more efficient[21,239]. A few years later it became apparent that such low-dimensional architecture exists in the entorhinal cortex.

When grid cells entered the research arena in 2005 (ref. 9), it was quite obvious that the dynamics proposed for localized firing in place cells might take place also in parahippocampal regions[91,151,239], as alluded to already by Samsonovitch and McNaughton[90]. In the first models proposed after the discovery of grid cells[91,151], cells were arranged on a matrix according to the phase of the grid. A bump of activity was formed when cells with similar phases were connected through excitatory connections, in the presence of global inhibition. Competitive network interactions led to multiple activity bumps[151], or toroidal connectivity caused a single bump that returned periodically to the same location[91]. Under certain conditions, in the presence of tonic excitatory input, a radius of inhibitory connectivity was sufficient to generate hexagonally patterned firing, without intrinsic excitatory connections[241–244].

Whether a path-integration-based attractor-network architecture exists in MEC remains to be determined, but there is indirect evidence for this possibility. First, correspondence between movement and displacement on the neural sheet can only be maintained so long as the participating grid cells have a common scale and orientation. Grid cells exist at a range of scales, suggesting that, to maintain the correspondence, grid cells must be organized in functionally independent grid modules, all with their own spacing and orientation[91,172]. Experimental evidence suggests that such a modular functional organization is indeed present[146,154]. A second observation consistent with a path-integration-dependent attractor architecture is the maintenance of a single grid-phase structure across environments, tasks and brain states[145,163,168,169], which would be expected if MEC neurons are organized as strongly interconnected networks in which external inputs recruit the same subset of neurons under a wide range of starting conditions. The strongest prediction of the attractor models, however, is perhaps that grid cells with similar grid phases have enhanced connectivity. Statistical analysis of firing patterns in simultaneously recorded grid cells confirm this prediction[245,246], but direct measurements of connections between functionally verified cell types are still missing.

Attractor models do not provide the only possible explanation of how grid patterns might be created. For several years, a competing class of models, based on properties of the hippocampal theta-frequency network rhythm[60–62], suggested that grid patterns were generated as a result of wave interference between a constant global theta oscillation and a velocity-controlled cell-specific theta oscillation[247–250]. The model can be traced back to O'Keefe and Recce's observation, in the early 1990s, that, as animals move through the place field of a place cell on a linear track, the spike times of the cell move forward across the cycle of background theta oscillations[251]. As the animal moves through the field, the theta phase of the spikes moves progressively forward also in space, and is in fact more strongly correlated with location than with time[251,252]. This observation suggested to O'Keefe and colleagues that position could be calculated from the interference pattern between the global

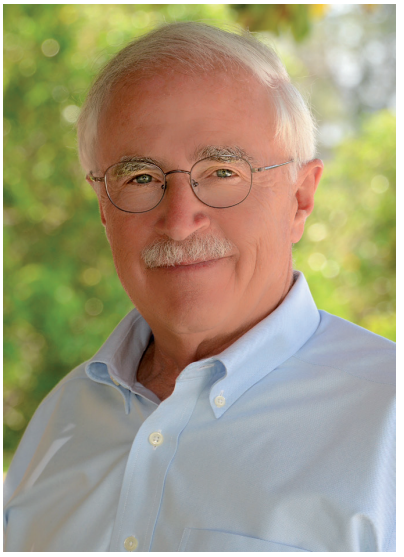
theta rhythm and a velocity-dependent oscillator specific to the cell. If position reflected peaks of the interference pattern, however, the firing positions should be periodic, which, for place cells, they were not. With the discovery of grid cells, the model was instantly revised and grid patterns were suggested to emerge from interference with velocity-controlled oscillators controlled by the projection of velocity in three directions separated by 60° intervals onto three separate dendrites[247–249]. Interference with the global oscillator led to a band-like spatial-activity pattern along each orientation, and the combination of bands led to a hexagonal pattern. The oscillatory interference models guided some of the most influential studies of grid formation, but in the end, accumulating evidence, such as the biophysical implausibility of independent dendritic oscillations[253], the sensitivity to period irregularity[254], the persistence of grid patterns in the absence of theta oscillations[219,255], the presence of a ramping depolarization, and the absence of a theta interference oscillation, in intracellular recordings from MEC cells[256,257], suggested that oscillatory interference is not the mechanism of the grid pattern. Yet phase precession is a reliable observation. Although it may not explain periodicity in grid cells, phase precession causes sequences of place cell activation to be replicated, in compressed format, within individual theta cycles, an effect that may be used by hippocampal circuits to store temporal sequences in addition to mere locations[252]. Indeed, as recognized by several investigators soon after phase precession was discovered[252,258,259], theta rhythm and phase precession may exist precisely to enable memory for spatial and temporal sequences.

The evidence against the oscillatory-interference model did not, however, rule out single-cell properties as determinants of the grid pattern. Kropff and Treves[24] showed how hexagonally patterned firing may arise through competitive Hebbian plasticity in a path-integration-independent manner in feedforward networks in which neurons undergo neuronal fatigue or adaptation. Because the emergence of grids in this model required many iterations, it was proposed that the adaptation mechanism contributed particularly to development of the network in young animals and that the coherence of phase and orientation relationships across environments was the result of recurrent connections that were added as the cortex matured[260]. Thus, competitive Hebbian plasticity offers an alternative mechanism for grid formation, although this mechanism may coexist with attractor-network architectures[261]. Regardless of mechanism, accounts of grid formation must consider not only intrinsic MEC dynamics but also how external inputs from the hippocampus[242], the medial septum[181,182], and locomotor[204–207,262] and head direction circuits[263] contribute to the emergence of grid patterns (**Box 1**).

Perspective

The search for a hippocampal positioning system began with the discovery of place cells in 1971. We have illustrated how the next few decades were characterized by attempts to find the determinants of spatially localized firing, with a focus on the sensory sources. As we entered the 1990s, the discovery of head direction cells and the turn to population dynamics prepared the field for more-targeted investigation of the circuit operations underlying place field formation and spatial mapping. The 1990s showed how ensembles of simultaneously recorded hippocampal neurons encoded functions that could not be read out from the activity of individual neurons. From around 2000, with increasing awareness that these ensembles likely extended beyond the hippocampus, investigators entered the entorhinal cortex, and an intricate circuit of grid cells and other specialized cell types was discovered there. The investigation of space has been brought to a new level, where it is possible to ask questions about how functions emerge through interactions within extended networks of heterogeneously connected cell types and subsystems.

While we will certainly learn more about the neural origins of spatial cognition during the years to come (**Box 1**), studies of spatial representation and navigation are informative about cortical functions in a wider sense. The ease with which spatial functions can be examined in the hippocampal formations of a number of mammals has made the study of the positioning system an area in which investigators pioneer the development and testing of sophisticated computational neural-network models. Few other areas of systems neuroscience have benefited so strongly from the interplay between computational and experimental neuroscience. Place cells and their entorhinal counterparts have helped open the cortex to studies of neural computation, allowing researchers to identify generic circuit motifs that may be expressed not only in the spatial circuits of the hippocampus and entorhinal cortex but across widespread regions of the brain. Almost 50 years after place cells were discovered, place cells and their parahippocampal counterparts have become one of the most powerful tools we have for understanding cortical computation and spatial mapping, and navigation may become one of the first cognitive functions to be understood in mechanistic terms.



Howard Eichenbaum (1947–2017). Few individuals have contributed more to the modern understanding of hippocampal memory function, with place cells as a key component, than Howard Eichenbaum, who sadly passed away, far too early, before the publication of this article. Photo credit: photographer Dan Kirksey, KDKC Photos, Escondito, CA.

IN MEMORIAM

In memoriam, Howard B. Eichenbaum (1947–2017). The field of hippocampal and memory research mourns the loss of our friend and colleague Howard, who passed away unexpectedly recently. Howard's contributions to the field were immense, both scientifically and in service. His research was mostly focused on one of the major aspects that we have explicitly not covered in this review: the role of the hippocampus in memory. Over the years, his position evolved from that of an unafraid and much-needed devil's advocate against the pure spatial map hypothesis towards what is now the general consensus view that spatial coding provides a foundation on top of which sensory and event-specific memory is superimposed, and he became a pioneer in the study of how time and temporal order also play a role. His thinking on hippocampal–cortical interactions in memory organization and control is beautifully summarized in his 2017 *Annual Review of Psychology* article[47].

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The authors declare no competing financial interests.

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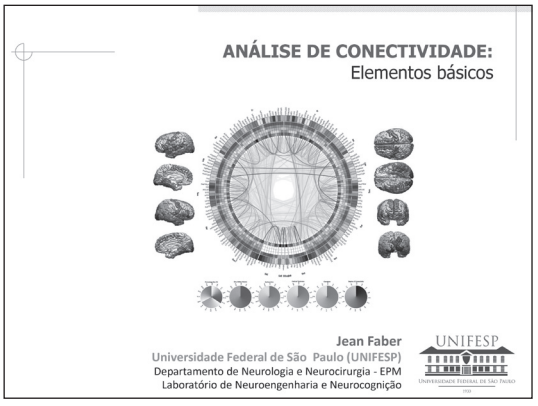
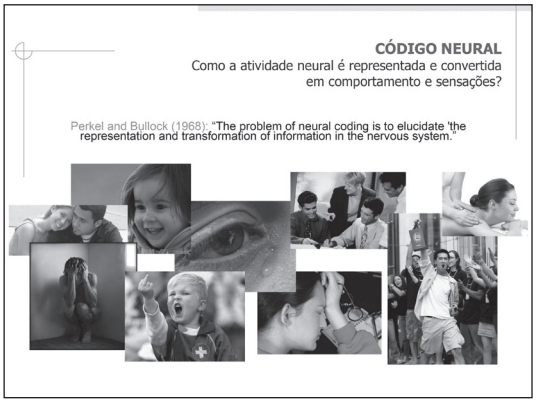
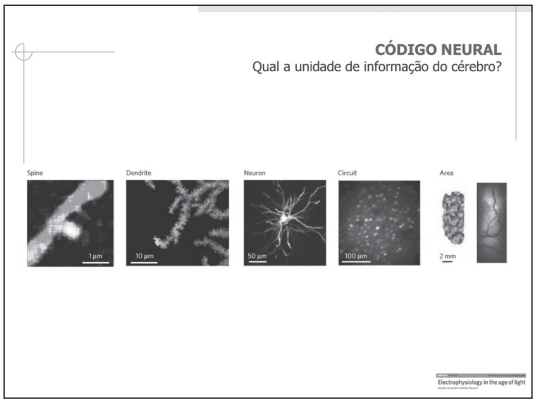
Jean Faber (Brazil)

THE 5 CRITICAL ELEMENTS OF A CONNECTOME: NODES, LINKS, TOPOLOGY, DYNAMICS AND MULTIPLEX SCALING







PROCESSAMENTO DE INFORMAÇÃO

Como construir um critério para representar e processar informação?

RECURSO | CRITÉRIO

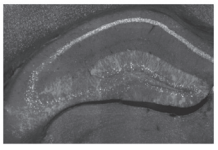
GENES, NEURONS, REGIONS — NUCLEOTÍDEOS, SPIKES, LFP, NEUROTRANSMISSORES

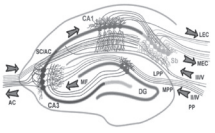
RULE — DETERMÍNISTCO, PROBABILÍSTICO

ACTING — EMOÇÕES, COGNIÇÃO, CONSCIÊNCIA

CÓDIGO NEURAL:

O critério de processamento neural depende do substrato?

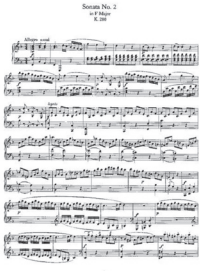




http://www.bristol.ac.uk/synaptic/pathways/

CÓDIGO NEURAL:

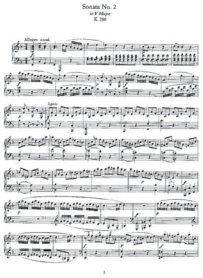
O critério de processamento neural depende do substrato?

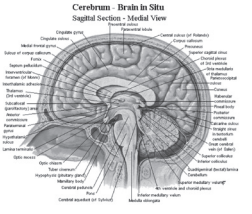


CÓDIGO NEURAL:

O critério de processamento neural depende do substrato?

O cérebro parece ter "muitos instrumentos"!





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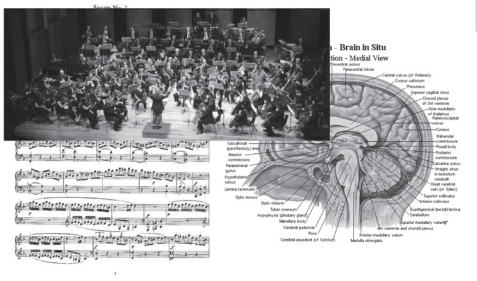
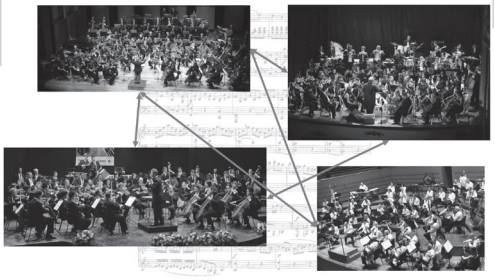


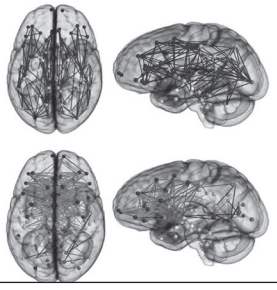
CÓDIGO NEURAL:
O critério de processamento neural depende do substrato?

O cérebro parece ter "muitas orquestras"!



CÓDIGO NEURAL E CONECTIVIDADE
O critério de processamento neural depende do substrato?

O cérebro parece ter "muitas orquestras"!

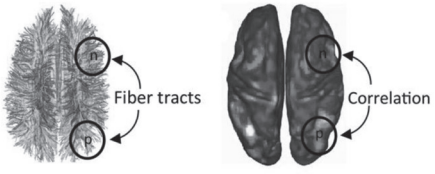


CÓDIGO NEURAL E CONECTIVIDADE
O critério de processamento neural depende do substrato?

CONNECTOMICS: Análise de conectividade neural

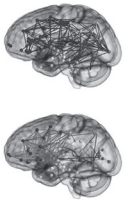
CONNECTIVITY

STRUCTURAL **FUNCTIONAL**



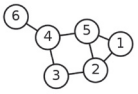
CONECTIVIDADE
Critério e representação do código neural?

Representação matemática: Teoria de Grafos



Um grafo é um par ordenado G(V,E), com V : {vértices} e E : {arestas}

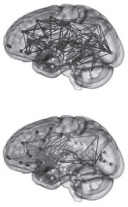
The Königsberg Bridge problem



V = {1, 2, 3, 4, 5, 6};
E = {{1, 2}, {1, 5}, {2, 3}, {2, 5}, {3, 4}, {4, 5}, {4, 6}}.

CONECTIVIDADE
Critério e representação do código neural?

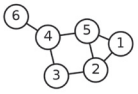
Representação matemática: Teoria de Grafos



Um grafo é um par ordenado G(V,E), com V : {vértices} e E : {arestas}

$$\begin{pmatrix} 2 & 1 & 0 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 1 & 1 \\ 1 & 1 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \end{pmatrix} = \mathbf{M}$$

Matriz de Adjacência



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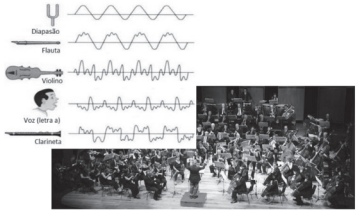
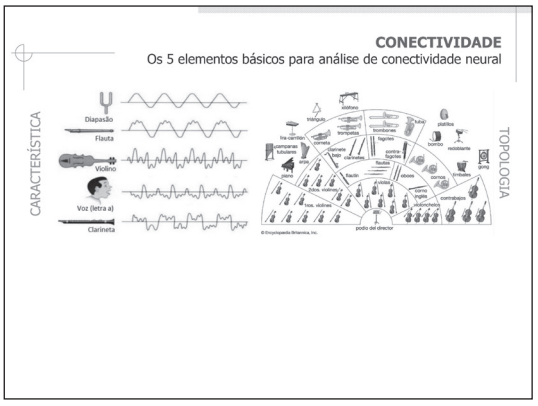
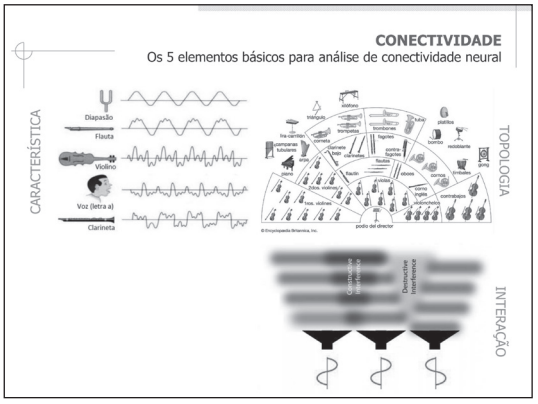
CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural

CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural

CARACTERÍSTICA




CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
CARACTERÍSTICA
Diapasão
Flauta
Violino
Voz (letra a)
Clarineta
TOPOLOGIA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Os 5 elementos básicos para análise de conectividade neural
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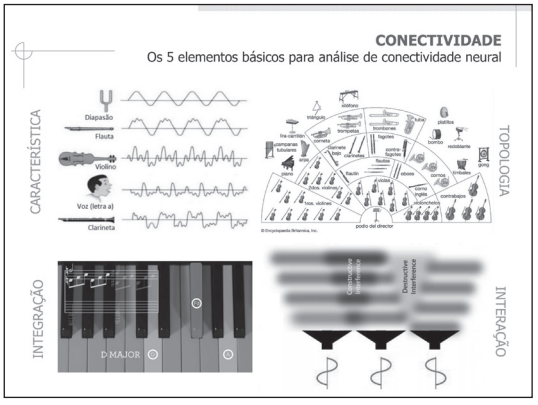
Diapasão
Flauta
Violino
Voz (letra a)
Clarineta
TOPOLOGIA
INTERAÇÃO


CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
CARACTERÍSTICA
Diapasão
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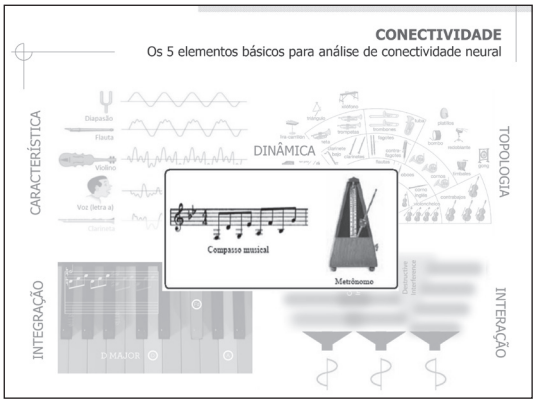
Voz (letra a)
Clarineta
TOPOLOGIA
INTEGRAÇÃO
D MAJOR
INTERAÇÃO


CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
CARACTERÍSTICA
Diapasão
Flauta
Violino
Voz (letra a)
Clarineta
DINÂMICA
Compasso musical
Metrônomo
TOPOLOGIA
INTEGRAÇÃO
D MAJOR
INTERAÇÃO

CONECTIVIDADE

Os 5 elementos básicos para análise de conectividade neural

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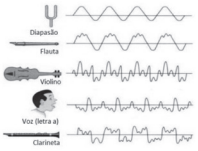
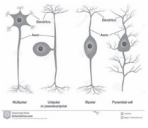


CONECTIVIDADE

Os 5 elementos básicos para análise de conectividade neural

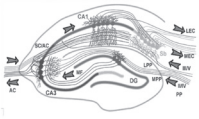
CARACTERÍSTICA



CONECTIVIDADE

Os 5 elementos básicos para análise de conectividade neural

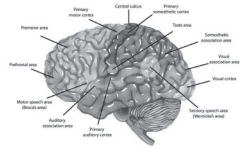
CARACTERÍSTICA



CONECTIVIDADE

Os 5 elementos básicos para análise de conectividade neural

CARACTERÍSTICA



CONECTIVIDADE

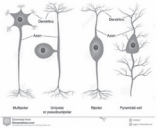
FRAMEWORK OF POSSIBLE BRAIN NODES		
STRUCTURAL NODES	SIGNALIZATION : FUNCTIONAL NODES	MEASUREMENT TECHNIQUES
	GENE EXPRESSION	NORTHERN BLOT, REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION, DNA MICROARRAY, RNA-SEQUENCING
	BIOMOLECULAR CELL SIGNILING INTRACELULAR POTENTIALS NEUROTRANSMITIONS IONIC CONCENTRATION	FLUORESCENCE CORRELATION SPECTROSCOPY, SHARP ELECTRODE, IMMUNOFLUORESCENCE, ION SELECTIVE ELECTRODE
	BIOMOLECULAR SIGNILING MEMBRANE AND INTRA/EXTRACELULAR POTENTIALS FLUORECENCE CELL IMAGING	SINGLE UNITY RECORDING, PATCH CLAMP, VOLTAGE AND CURRENT CLAMP, FLUORECENCE IMAGING
	BIOMOLECULAR SIGNILING INTRA/EXTRACELULAR POTENTIALS FLUORECENCE CELL EXPRESSION METABOLIC CELL EXPRESSION	MULTIUNITY RECORDING, MICROELETRODES, FLUORECENCE IMAGING, IMUNOHISTOCHEMISTRY
	INTRA/EXTRACELULAR POTENTIALS ELECTRIC AND MAGNETIC BIOPOTENTIALS METABOLIC EXPRESSION	MULTIUNITY RECORDING, INVASIVE PROBES, EEG, ECoG, fNIRS, fMRI, PET, MEG

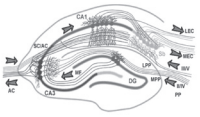
CONECTIVIDADE

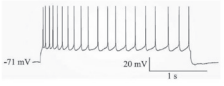
Os 5 elementos básicos para análise de conectividade neural

CARACTERÍSTICA (ANATO-ELETROFISIOLÓGICA):

CANDIDATOS PARA REPRESENTAÇÃO DO CÓDIGO NEURAL

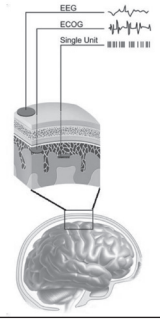


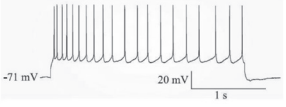




CÓDIGO NEURAL:

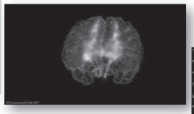
Existiria alguma "partitura" como liguagem comum?

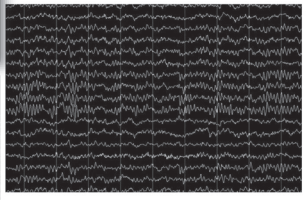


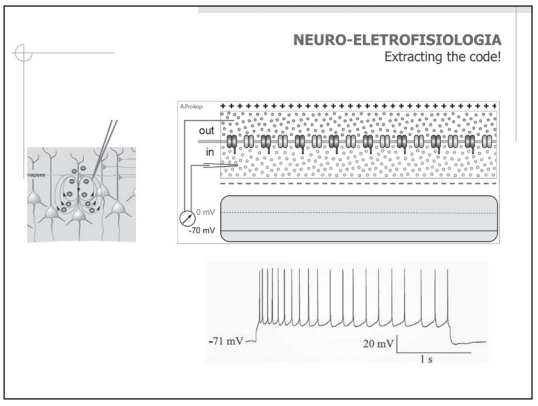


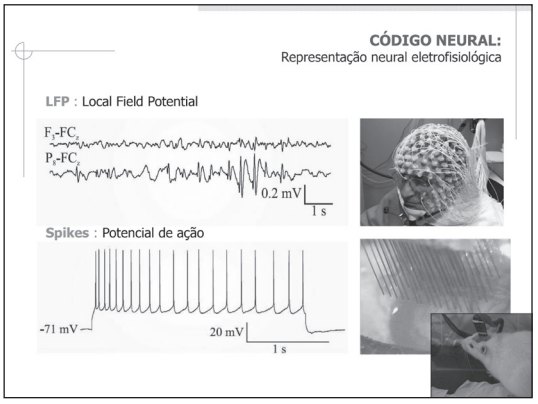
NEURO-ELETROFISIOLOGIA

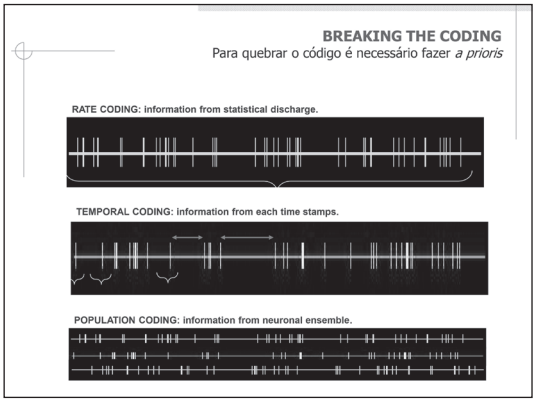
Extracting the code!

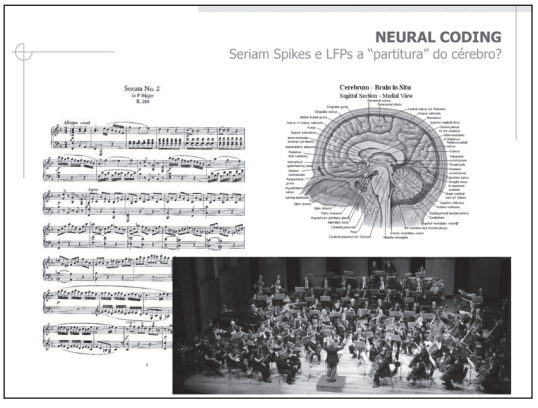


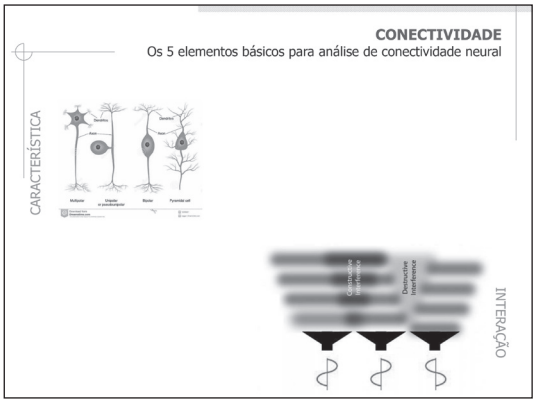


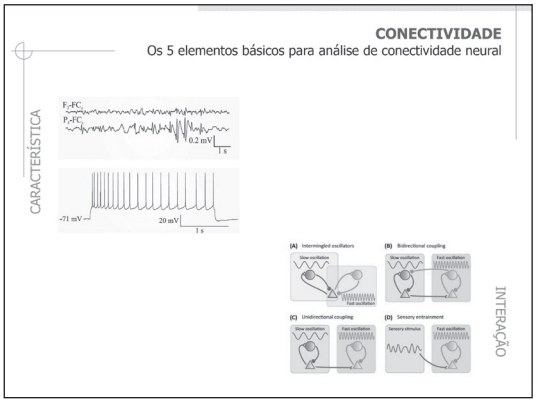

NEURO-ELETROFISIOLOGIA
Extracting the code!
out
in
0 mV
-70 mV
-71 mV
20 mV
1 s


CÓDIGO NEURAL:
Representação neural eletrofisiológica
LFP : Local Field Potential
0.2 mV
1 s
Spikes : Potencial de ação
-71 mV
20 mV
1 s


BREAKING THE CODING
Para quebrar o código é necessário fazer *a prioris*
RATE CODING: information from statistical discharge.
TEMPORAL CODING: information from each time stamps.
POPULATION CODING: information from neuronal ensemble.


NEURAL CODING
Seriam Spikes e LFPs a "partitura" do cérebro?
Sonata No. 2
in F Major
K. 280
Cerebrum - Brain in Situ
Sagittal Section - Medial View


CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
CARACTERÍSTICA
INTERAÇÃO


CONECTIVIDADE
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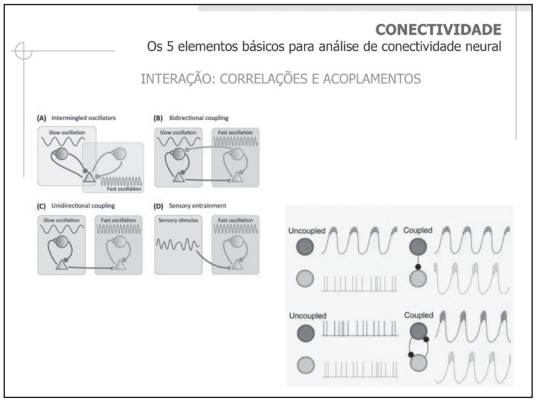
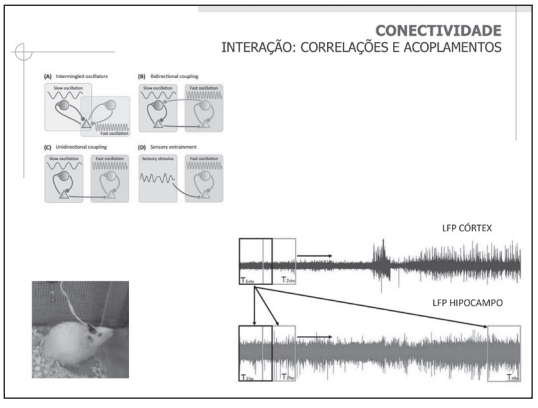
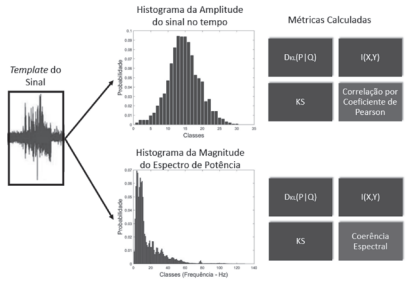
CARACTERÍSTICA
INTERAÇÃO


CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
INTERAÇÃO: CORRELAÇÕES E ACOPLAMENTOS
(A) Intermingled oscillators
(B) Bidirectional coupling
(C) Unidirectional coupling
(D) Sensory entrainment
Uncoupled
Coupled


CONECTIVIDADE
INTERAÇÃO: CORRELAÇÕES E ACOPLAMENTOS
LFP CÓRTEX
LFP HIPOCAMPO

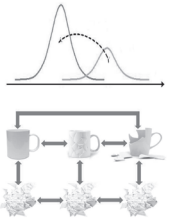
CONECTIVIDADE
INTERAÇÃO: CORRELAÇÕES E ACOPLAMENTOS

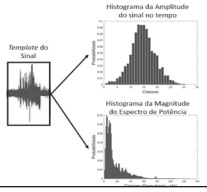


CONECTIVIDADE
INTERAÇÃO: CORRELAÇÕES E ACOPLAMENTOS

*** Coeficiente de Pearson:** Grau da relação entre duas variáveis quantitativas

$$\rho = \frac{cov(X,Y)}{\sqrt{var(X).var(Y)}}$$

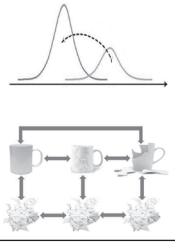


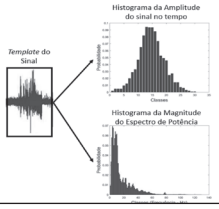


CONECTIVIDADE
INTERAÇÃO: CORRELAÇÕES E ACOPLAMENTOS

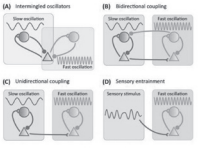
*** Kullback-Leibler:** mede o custo de levar uma distribuição de probabilidade P a Q:

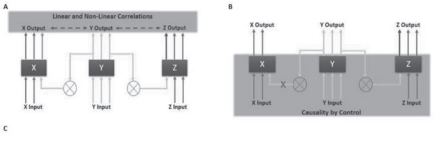
$$D_{KL}(P|Q) = \sum_{i}^{n} p(x_i)\, log_b\left(\frac{p(x_i)}{q(x_i)}\right)$$





CONECTIVIDADE
INTERAÇÃO: CORRELAÇÕES E ACOPLAMENTOS





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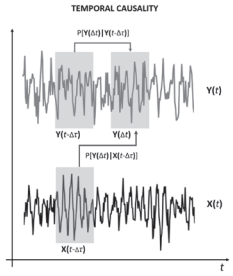
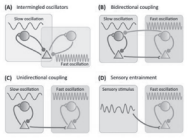
INTERAÇÃO: CORRELAÇÕES E ACOPLAMENTOS





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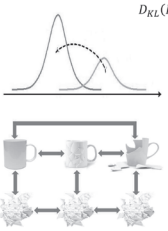

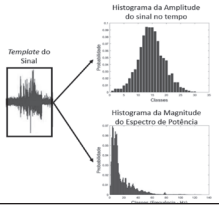

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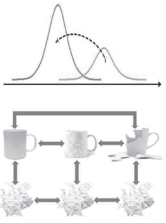
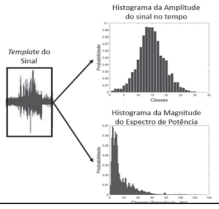


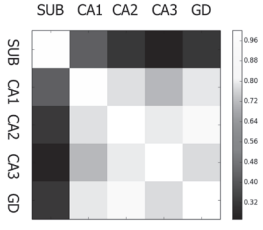


CONECTIVIDADE
Construindo topologias

TOPOLOGIA DE REDE (FUNCIONAL)

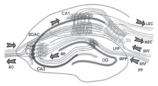
$$D_{KL}(P||Q) = \sum_{x \in X} P(x) log \frac{P(x)}{Q(x)}$$

$$COR = \frac{\sum_{i=1}^{N}\sum_{m=1}^{M}(x_{im}-\bar{x})(y_{im}-\bar{y})}{\sqrt{\sum_{i=1}^{N}\sum_{m=1}^{M}(x_{im}-\bar{x})^2}\sqrt{\sum_{i=1}^{N}\sum_{m=1}^{M}(y_{im}-\bar{y})^2}},$$



http://artedosdados.blogspot.com.br/2015/01/matriz-de-correlacao-em-python.html

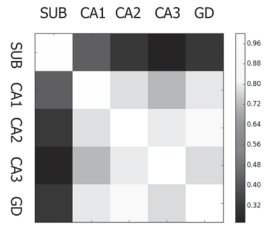
CONECTIVIDADE
Construindo topologias



TOPOLOGIA DE REDE (FUNCIONAL)

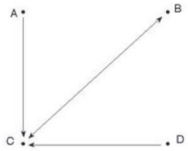
$$D_{KL}(P||Q) = \sum_{x \in X} P(x) log \frac{P(x)}{Q(x)}$$

$$COR = \frac{\sum_{i=1}^{N}\sum_{m=1}^{M}(x_{im}-\bar{x})(y_{im}-\bar{y})}{\sqrt{\sum_{i=1}^{N}\sum_{m=1}^{M}(x_{im}-\bar{x})^2}\sqrt{\sum_{i=1}^{N}\sum_{m=1}^{M}(y_{im}-\bar{y})^2}},$$



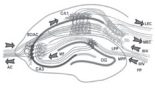
http://artedosdados.blogspot.com.br/2015/01/matriz-de-correlacao-em-python.html

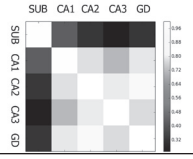
CONECTIVIDADE
Construindo topologias



	A	B	C	D
A	0	0	1	0
B	0	0	1	0
C	0	1	0	0
D	0	0	1	0

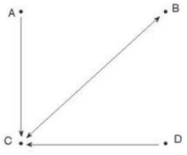
A	C	1
B	C	1
C	B	1
D	C	1





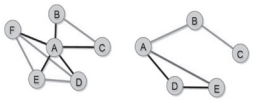
M. Kaiser, A Tutorial in Connectome Analysis, 2016.

CONECTIVIDADE
Construindo topologias



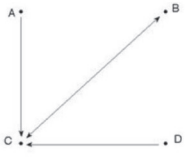
	A	B	C	D
A	0	0	1	0
B	0	0	1	0
C	0	1	0	0
D	0	0	1	0

A	C	1
B	C	1
C	B	1
D	C	1



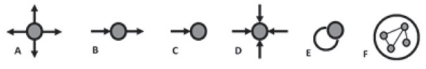
M. Kaiser, A Tutorial in Connectome Analysis, 2016.

CONECTIVIDADE
DIFERENTES TIPOS DE NÓS...

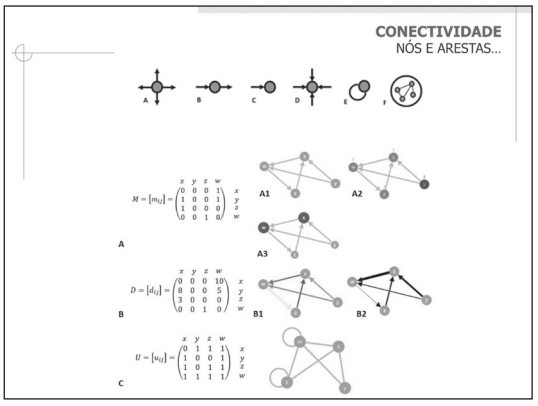


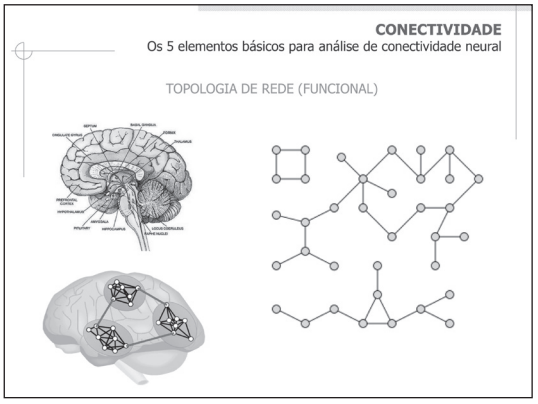
	A	B	C	D
A	0	0	1	0
B	0	0	1	0
C	0	1	0	0
D	0	0	1	0

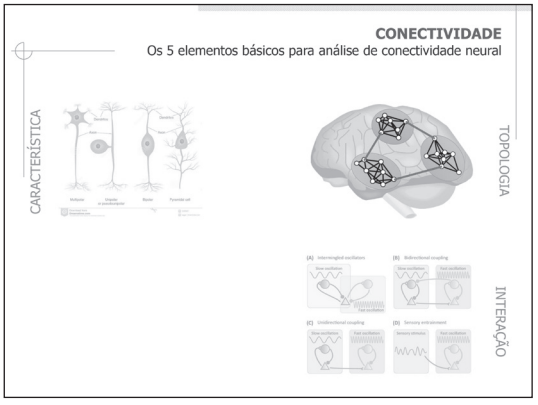
A	C	1
B	C	1
C	B	1
D	C	1

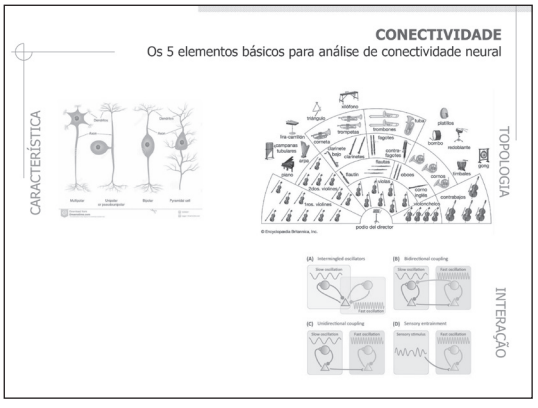


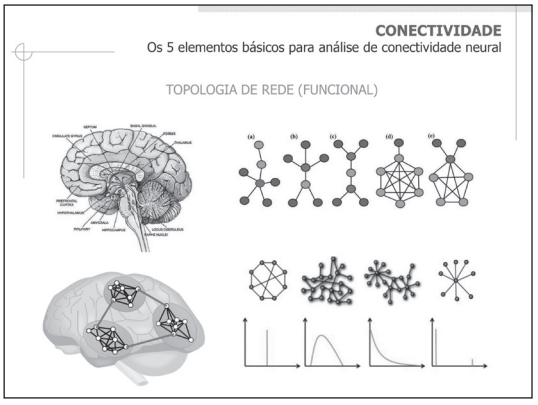
M. Kaiser, A Tutorial in Connectome Analysis, 2016.

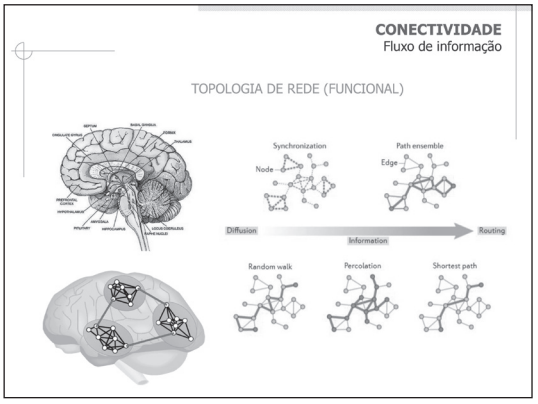

CONECTIVIDADE
NÓS E ARESTAS...
A
B
C
D
E
F
A1
A2
A3
B1
B2
A
B
C

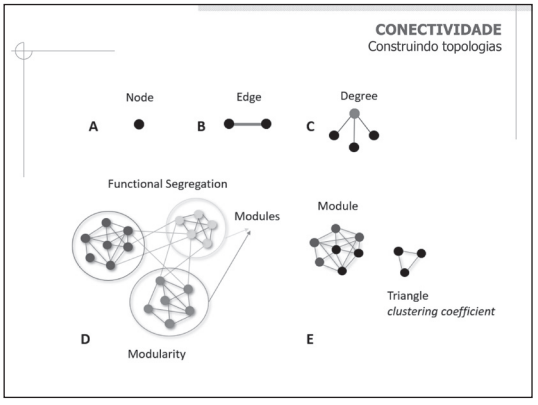

CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
TOPOLOGIA DE REDE (FUNCIONAL)

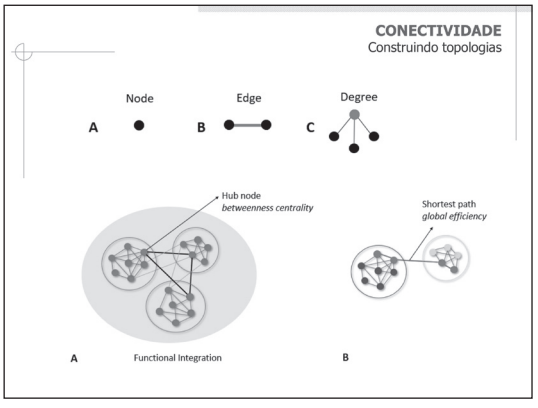

CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
CARACTERÍSTICA
TOPOLOGIA
INTERAÇÃO

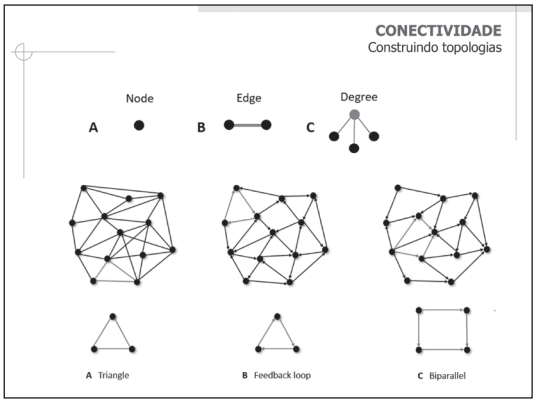

CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
CARACTERÍSTICA
TOPOLOGIA
INTERAÇÃO

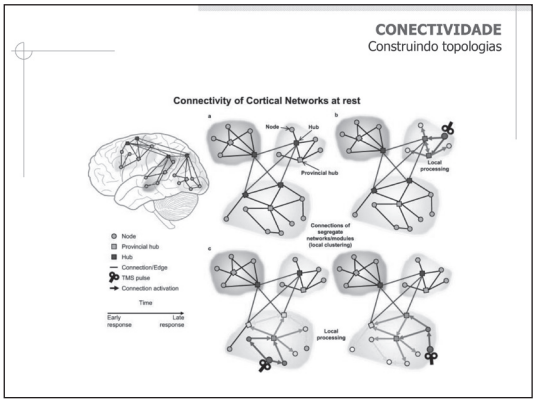

CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
TOPOLOGIA DE REDE (FUNCIONAL)

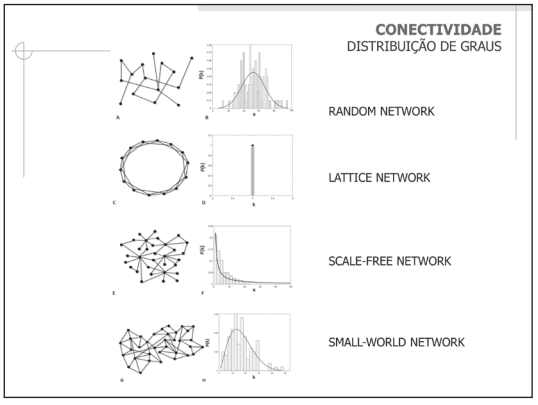

CONECTIVIDADE
Fluxo de informação
TOPOLOGIA DE REDE (FUNCIONAL)
Synchronization
Node
Path ensemble
Edge
Diffusion
Information
Routing
Random walk
Percolation
Shortest path

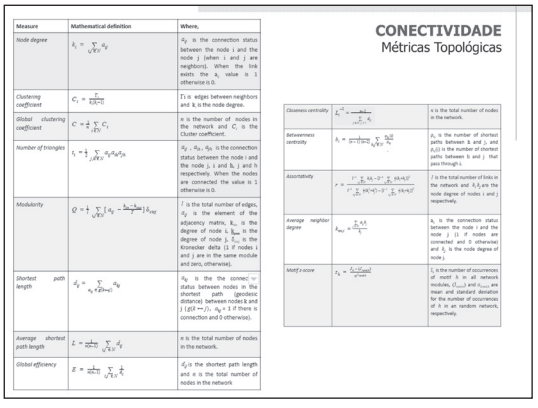

CONECTIVIDADE
Construindo topologias
Node
Edge
Degree
A
B
C
Functional Segregation
Modules
Module
Triangle
clustering coefficient
D
Modularity
E


CONECTIVIDADE
Construindo topologias
Node
Edge
Degree
A
B
C
Hub node
betweenness centrality
Shortest path
global efficiency
A
Functional Integration
B





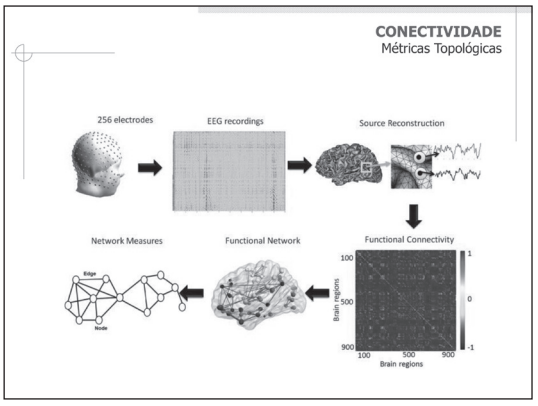


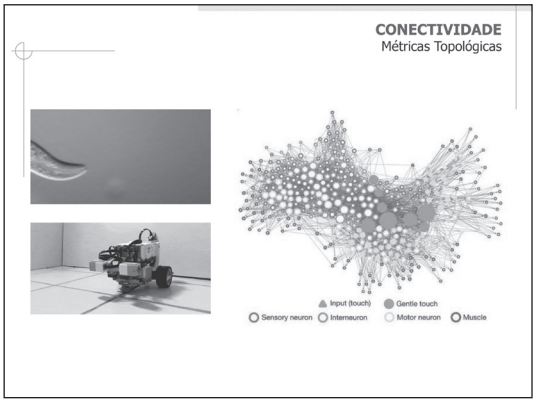


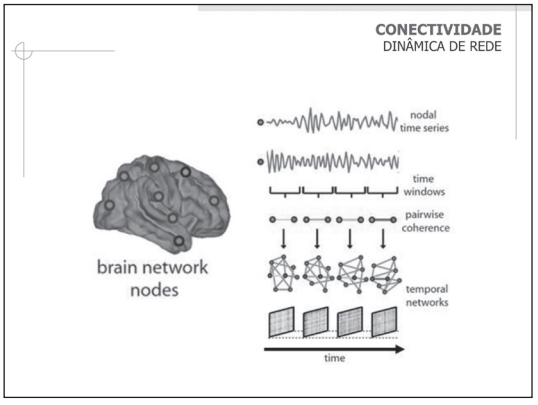
Measure	Mathematical definition	Where,
Node degree	$k_i = \sum_{j \in N} a_{ij}$	a_{ij} is the connection status between the node i and the node j (when i and j are neighbors). When the link exists the a_{ij} value is 1 otherwise is 0.
Clustering coefficient	$C_i = \frac{\Gamma_i}{k_i(k_i-1)}$	Γ_i is edges between neighbors and k_i is the node degree.
Global clustering coefficient	$C = \frac{1}{n}\sum_{i \in N} C_i$	n is the number of nodes in the network and C_i is the Cluster coefficient.
Number of triangles	$t_i = \frac{1}{2}\sum_{j,h \in N} a_{ij} a_{jh} a_{hi}$	a_{ij}, a_{jh}, a_{hi} is the connection status between the node i and the node j, i and h, j and h respectively. When the nodes are connected the value is 1 otherwise is 0.
Modularity	$Q = \frac{1}{l}\sum_{i,j \in N}\left[a_{ij} - \frac{k_i k_j}{l}\right]\delta_{M_i M_j}$	l is the total number of edges, a_{ij} is the element of the adjacency matrix, $k_{i\_}$ is the degree of node i, $k_{j\_}$ is the degree of node j, $\delta_{\_}$ is the Kronecker delta (1 if nodes i and j are in the same module and zero, otherwise).
Shortest path length	$d_{ij} = \sum_{n_i \in P(i,j)-1} a_{ij}$	d_{ij} is the the connec-tion status between nodes in the shortest path (geodesic distance) between nodes i and j $\{g(i \leftrightarrow j)\}$, $a_{ij} = 1$ if there is connection and 0 otherwise).
Average shortest path length	$L = \frac{1}{n(n-1)}\sum_{i \neq j \in N} d_{ij}$	n is the total number of nodes in the network.
Global efficiency	$E = \frac{1}{n(n-1)}\sum_{i \neq j \in N}\frac{1}{d_{ij}}$	d_{ij} is the shortest path length and n is the total number of nodes in the network.

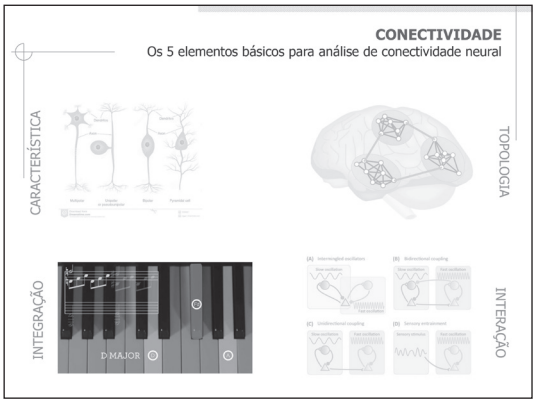
CONECTIVIDADE
Métricas Topológicas

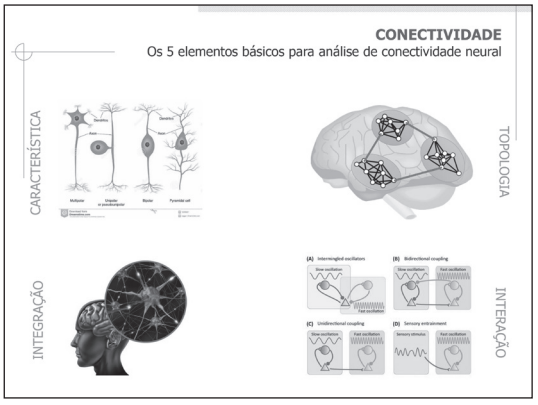
Closeness centrality	$C_i^{-1} = \frac{n-1}{\sum_{j \in N, j \neq i} d_{ij}}$	n is the total number of nodes in the network.
Betweenness centrality	$b_i = \frac{1}{(n-1)(n-2)}\sum_{h \neq i \neq j \in N}\frac{p_{hj}(i)}{p_{hj}}$	p_{hj} is the number of shortest paths between h and j, and $p_{hj}(i)$ is the number of shortest paths between h and j that pass through i.
Assortativity	$r = \frac{l^{-1}\sum_i k_i k_j - l^{-1}\sum_i \frac{1}{2}(k_i + k_j)^2}{l^{-1}\sum_i \frac{1}{2}(k_i^2 + k_j^2) - l^{-1}\sum_i \frac{1}{2}(k_i + k_j)^2}$	l is the total number of links in the network and k_i, k_j are the node degree of nodes i and j respectively.
Average neighbor degree	$k_{nn,i} = \frac{\sum_j a_{ij} k_j}{k_i}$	a_{ij} is the connection status between the node i and the node j (1 if nodes are connected and 0 otherwise) and k_i is the node degree of node i.
Motif z-score	$z_k = \frac{f_k - \langle f_{rand} \rangle}{\sigma_{rand}}$	f_k is the number of occurrences of motif k in all network modules, $\langle f_{rand} \rangle$ and σ_{rand} are mean and standard deviation for the number of occurrences of k in an random network, respectively.

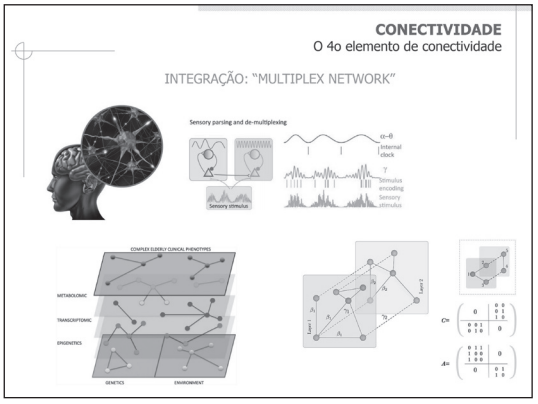

CONECTIVIDADE
Métricas Topológicas
256 electrodes
EEG recordings
Source Reconstruction
Network Measures
Functional Network
Functional Connectivity
Edge
Node
Brain regions
Brain regions

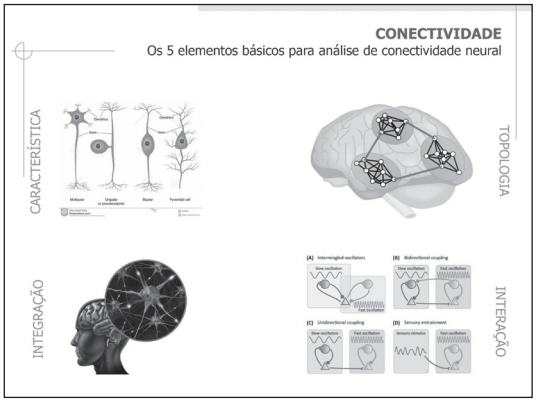

CONECTIVIDADE
Métricas Topológicas
Input (touch)
Gentle touch
Sensory neuron
Interneuron
Motor neuron
Muscle

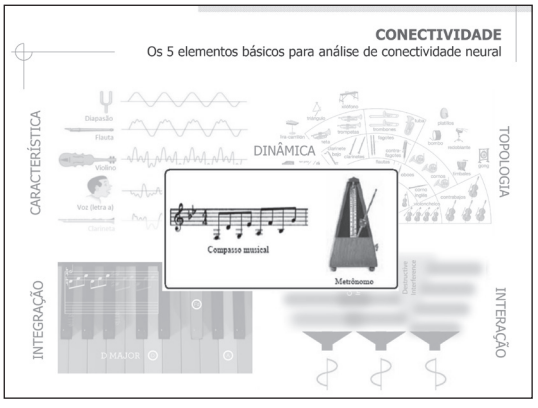

CONECTIVIDADE
DINÂMICA DE REDE
nodal time series
time windows
pairwise coherence
temporal networks
time
brain network nodes


CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
CARACTERÍSTICA
TOPOLOGIA
INTEGRAÇÃO
INTERAÇÃO
D MAJOR


CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
CARACTERÍSTICA
TOPOLOGIA
INTEGRAÇÃO
INTERAÇÃO


CONECTIVIDADE
O 4o elemento de conectividade
INTEGRAÇÃO: "MULTIPLEX NETWORK"
Sensory parsing and de-multiplexing
COMPLEX ELDERLY CLINICAL PHENOTYPES
METABOLOMIC
TRANSCRIPTOMIC
EPIGENETICS
GENETICS
ENVIRONMENT

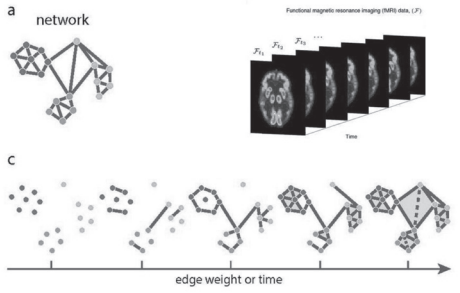

CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
CARACTERÍSTICA
TOPOLOGIA
INTEGRAÇÃO
INTERAÇÃO


CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural
CARACTERÍSTICA
TOPOLOGIA
INTEGRAÇÃO
INTERAÇÃO
Diapasão
Flauta
Violino
Voz (letra a)
Clarinete
DINÂMICA
Compasso musical
Metrônomo
D-MAJOR

CONECTIVIDADE

Os 5 elementos básicos para análise de conectividade neural

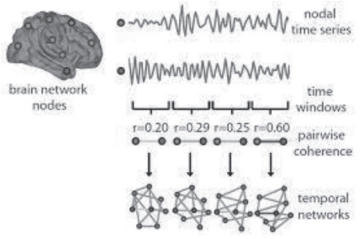
DINÂMICA DE CONECTIVIDADE



CONECTIVIDADE

Os 5 elementos básicos para análise de conectividade neural

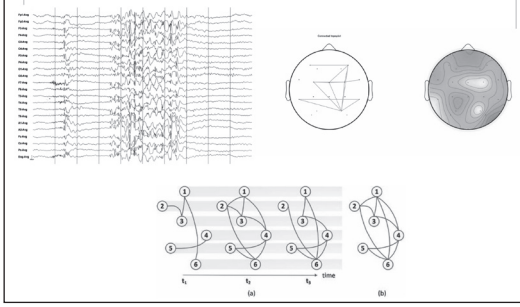
DINÂMICA DE CONECTIVIDADE



CONECTIVIDADE

Os 5 elementos básicos para análise de conectividade neural

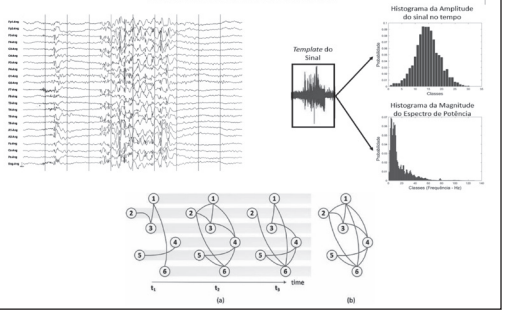
DINÂMICA DE CONECTIVIDADE



CONECTIVIDADE

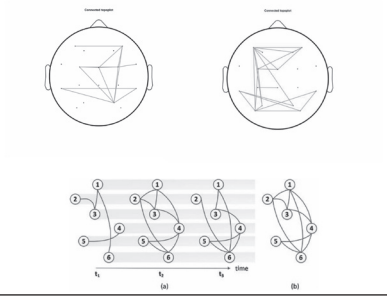
Os 5 elementos básicos para análise de conectividade neural

DINÂMICA DE CONECTIVIDADE



CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural

DINÂMICA DE CONECTIVIDADE

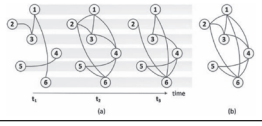


CONECTIVIDADE
Os 5 elementos básicos para análise de conectividade neural

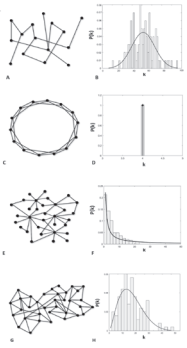
DINÂMICA DE CONECTIVIDADE

$$\rho = \frac{cov(X,Y)}{\sqrt{var(X).var(Y)}}$$

$$D_{KL}(P||Q) = \sum_{x \in X} P(x) log \frac{P(x)}{Q(x)}$$



CONECTIVIDADE
DISTRIBUIÇÃO DE GRAUS



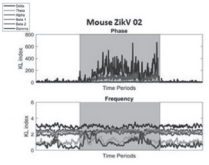
RANDOM NETWORK

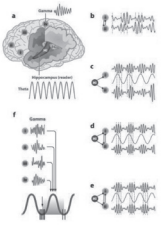
LATTICE NETWORK

SCALE-FREE NETWORK

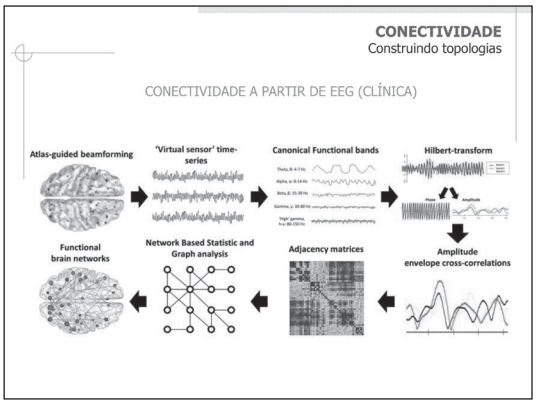
SMALL-WORLD NETWORK

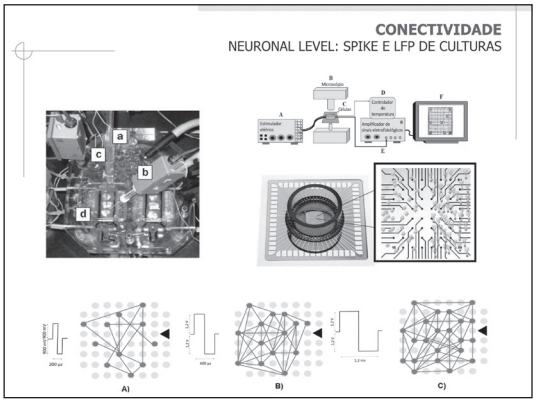
INTERFERÊNCIA ⇔ COERÊNCIA
"O segredo do universo está na fase!"

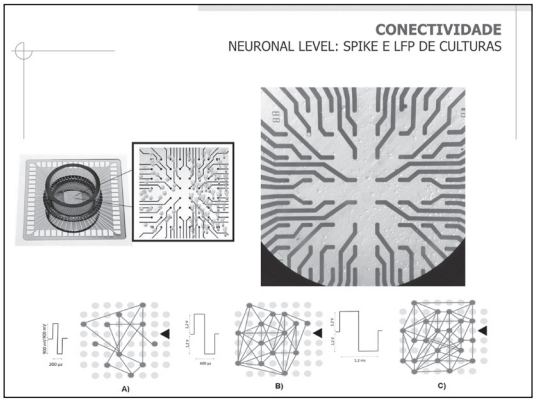


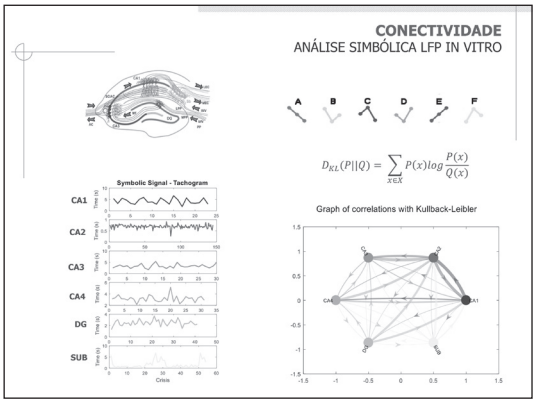


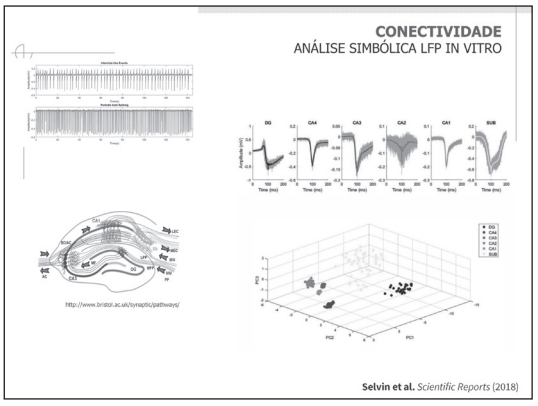
Buzsáki: Annu. Rev. Neurosci. 2012.

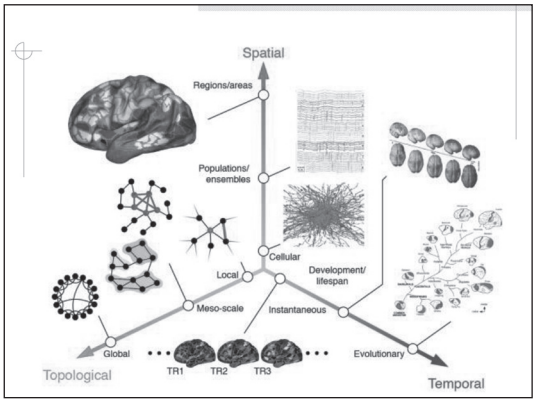

CONECTIVIDADE
Construindo topologias
CONECTIVIDADE A PARTIR DE EEG (CLÍNICA)
Atlas-guided beamforming
'Virtual sensor' time-series
Canonical Functional bands
Hilbert-transform
Phase
Amplitude
Amplitude envelope cross-correlations
Adjacency matrices
Network Based Statistic and Graph analysis
Functional brain networks


CONECTIVIDADE
NEURONAL LEVEL: SPIKE E LFP DE CULTURAS
A)
B)
C)

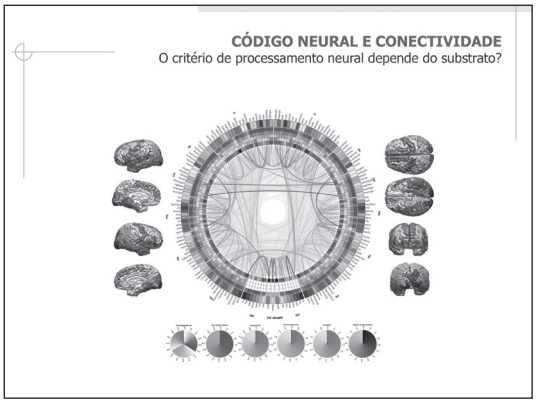

CONECTIVIDADE
NEURONAL LEVEL: SPIKE E LFP DE CULTURAS
A)
B)
C)


CONECTIVIDADE
ANÁLISE SIMBÓLICA LFP IN VITRO
Symbolic Signal - Tachogram
CA1
CA2
CA3
CA4
DG
SUB
Crisis
$D_{KL}(P||Q) = \sum_{x \in X} P(x) log \frac{P(x)}{Q(x)}$
Graph of correlations with Kullback-Leibler


CONECTIVIDADE
ANÁLISE SIMBÓLICA LFP IN VITRO
http://www.bristol.ac.uk/synaptic/pathways/
Selvin et al. Scientific Reports (2018)


Spatial
Regions/areas
Populations/
ensembles
Cellular
Local
Development/
lifespan
Meso-scale
Instantaneous
Global
Evolutionary
TR1
TR2
TR3
Topological
Temporal


Reseachers :
Jean Faber
Guillem Via
Students:
Karina Rodrigues, Ana Contier
Noemi Araujo, Priscila, Daniel Leal,
João Vitor, Lucas Shinoda
Thaís Cardoso, João Luiz
jeanfaber@gmail.com
CNPq
Conselho Nacional de Desenvolvimento
Científico e Tecnológico


CÓDIGO NEURAL E CONECTIVIDADE
O critério de processamento neural depende do substrato?

Philipe Mendonça (UK)

IMAGING PRESYNAPTIC ACTIVITY

UCL

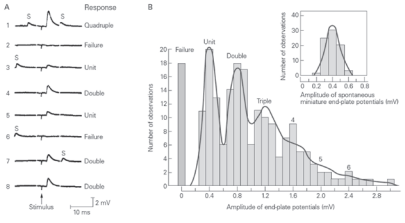
Imaging presynaptic activity in individual boutons

Philipe Mendonça

Institute of Neurology
University College London

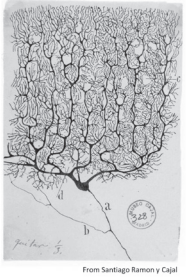
LASSE, March 2019

Principles of synaptic transmission



Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S. A., & Hudspeth, A. . (2013). *Principles of Neural Science* (5th ed.). McGraw-Hill.

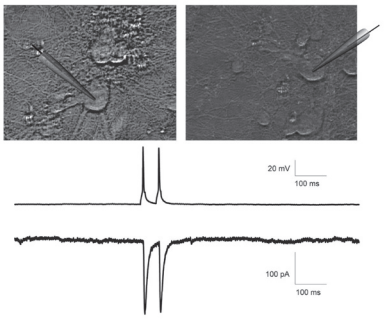
Synaptic transmission



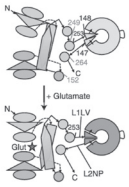
From Santiago Ramon y Cajal

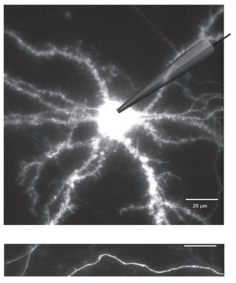
From Greg Dunn

Synaptic transmission



iGluSnFR overview





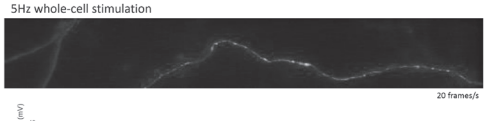
General approach

- Magnetofection at 5 DIV
- Patch-clamp at 15-20 DIV
- 5Hz whole-cell stimulation
- Image axonal arbour
- 137x20 µm (512x75 pixels), ~ 6ms per frame

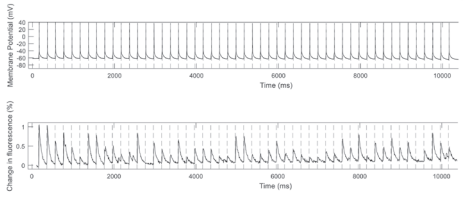
Marvin JS, Borghuis BG, Tian L, Cichon J, Harnett MT, Akerboom J, Gordus A, Renninger SL, Chen T-W, Bargmann CI, Orger MB, Schreiter ER, Demb JB, Gan W-B, Hires SA & Looger LL (2013). An optimized fluorescent probe for visualizing glutamate neurotransmission. *Nat Methods* **10**, 162–170.

Imaging synaptic activity

5Hz whole-cell stimulation

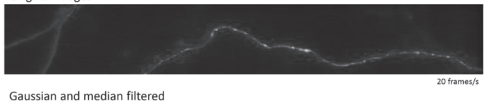


20 frames/s



iGluSnFR: detecting active boutons

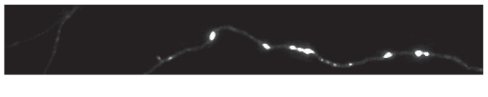
Original images



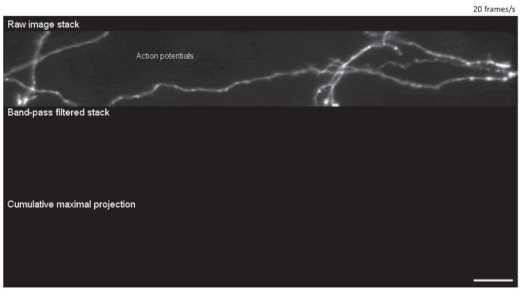
20 frames/s

Gaussian and median filtered

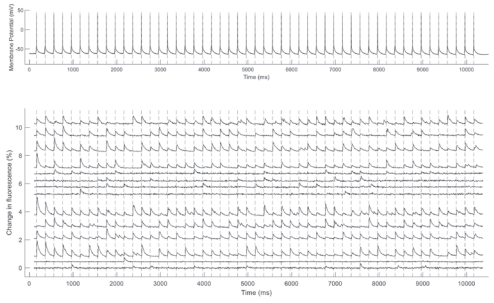
Maximal projection



iGluSnFR: detecting active boutons

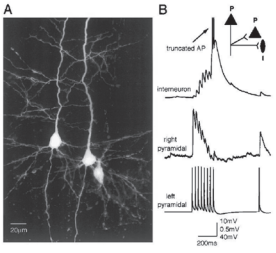


Vesicle release profile of a population of synapses



- Synapses (from the same axonal arbor) are heterogenous
- Vesicle release can - and often will - fail

Functional role of a heterogeneous vesicular release probability

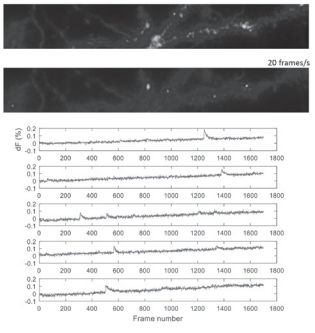


Low P_r
- Short-term potentiation
- High-pass filter

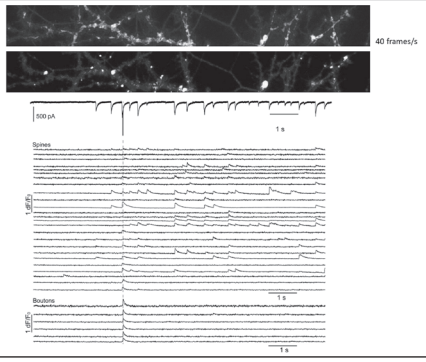
High P_r
- Short-term depression
- Low-pass filter

Markram, H., Wang, Y., & Tsodyks, M. (1998). Differential signaling via the same axon of neocortical pyramidal neurons. *PNAS*, *24*(1), 99–119.

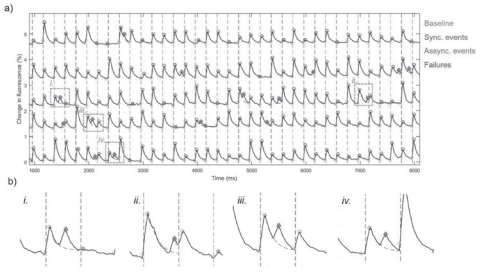
Detection of spontaneous release in individual spines

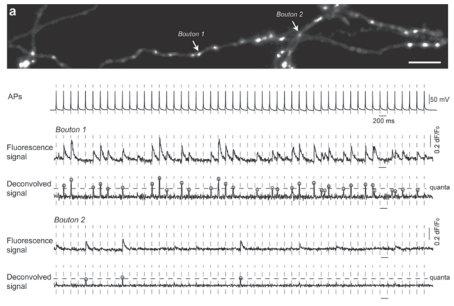


Synaptic integration in a neuronal segment (dendrite + axon)

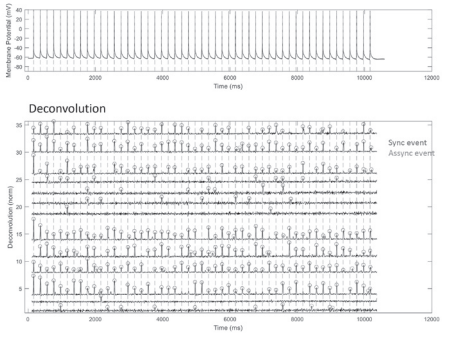


Detection of synchronous and asynchronous events

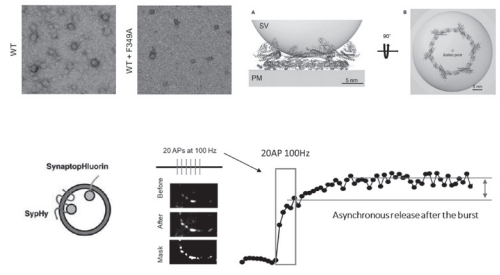




Detection of synchronous and asynchronous events

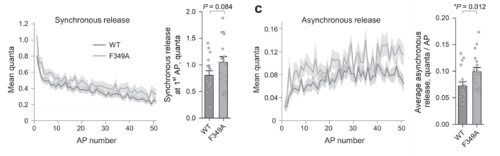


Application: Regulation of neurotransmission by synaptotagmin 1 oligomerisation



Miesenböck G, De Angelis DA & Rothman JE (1998). Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins. Nature 394, 192–195.
Rothman JE, Krishnakumar SS, Grushin K, Pincet F (2017) Hypothesis – buttressed rings assemble, clamp, and release SNAREpins for synaptic transmission. FEBS Lett 591:3459–3480.
Zanetti MN, Bello OD, Wang J, Coleman J, Cai Y, Sindelar CV , Rothman JE, Krishnakumar SS (2016) Ring-like oligomers of synaptotagmins and related C2 domain proteins. Elife 5:1–15.

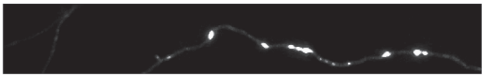
Application: Regulation of neurotransmission by synaptotagmin 1 oligomerisation



Tagliatti et al., unpublished

Detecting sites of synchronous and asynchronous events

Detecting synchronous and asynchronous events in the entire ROI



Synchronous release
Asynchronous release

10 frames/s

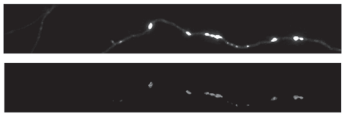
Spatial distribution of synchronous and asynchronous events

Caveats

- Slow fluorescence decay limits the frequency of stimulation
- Light intensity limits period of exposure (photodamage)
- Synapses with very low P_r may be not taken into account (<0.02)

Perspectives

- Increase temporal resolution:
  - New CMOS camera (Prime95b)
  - iGluSnFR with higher Kd (35 uM)
- Colocalization of synaptic properties and presynaptic proteins (Syt1 and Syt7)



- Dual channel recording: both Ca^{2+} influx and glutamate release (Cal590 and iGluSnFR)

Take home messages

- New imaging techniques provide an ever increasing temporal resolution and s/n
- Electrophysiology + fast live-imaging = optimal spatial and temporal resolution
- Synapses are heterogeneous, and for a good reason

Thank you!

UCL

Prof Kirill Volynski (University College London)
Dr Erica Taggliatti (University College London)

The Physiological Society

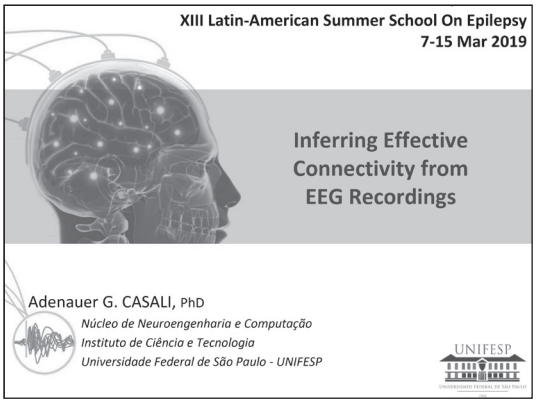
CAMBRIDGE PHILOSOPHICAL SOCIETY

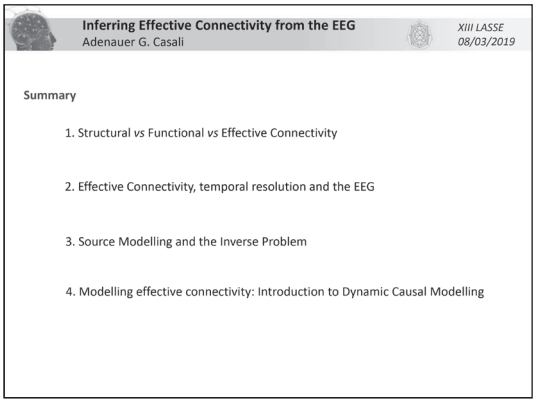
Jean Gotman (Canada)

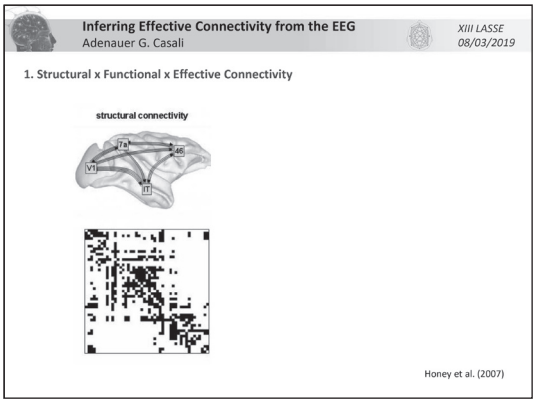
STRENGTH AND WEAKNESSES OF SOME BASIC METHODS: CORRELATION, COHERENCE, GRANGER CASUALITY

Adenauer Casali (Brazil)

INFERRING EFFECTIVE CONNECTIVITY FROM EEG RECORDINGS



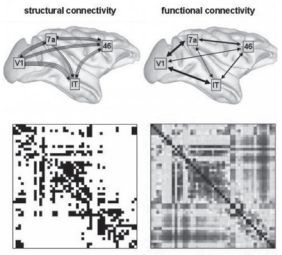




Inferring Effective Connectivity from the EEG
Adenauer G. Casali

XIII LASSE
08/03/2019

1. Structural x Functional x Effective Connectivity



Honey et al. (2007)

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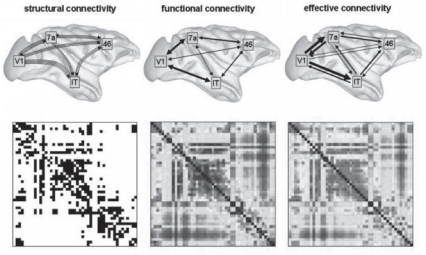
Adenauer G. Casali

XIII LASSE
08/03/2019

1. Structural x Functional x Effective Connectivity



Honey et al. (2007)

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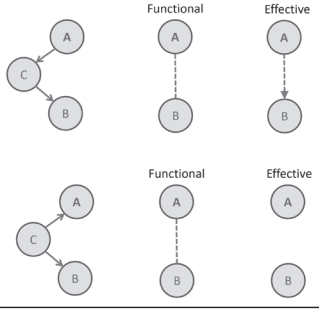
XIII LASSE
08/03/2019

1. Structural x Functional x Effective Connectivity



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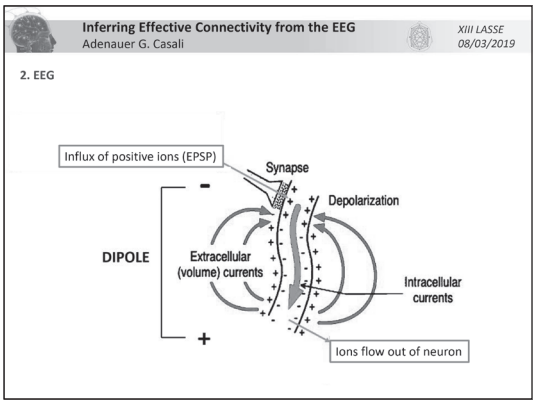
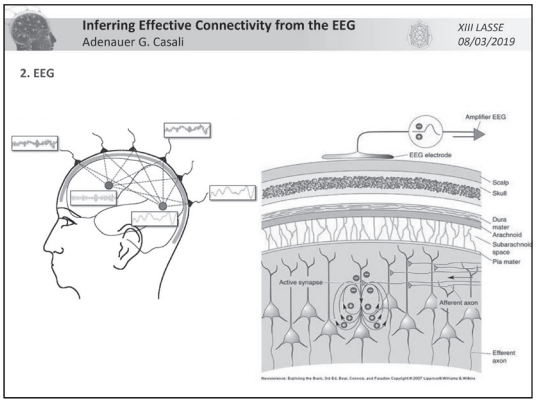
2. EEG

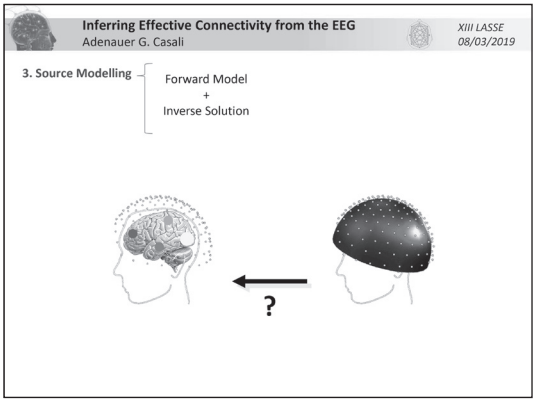
Non-invasiveness

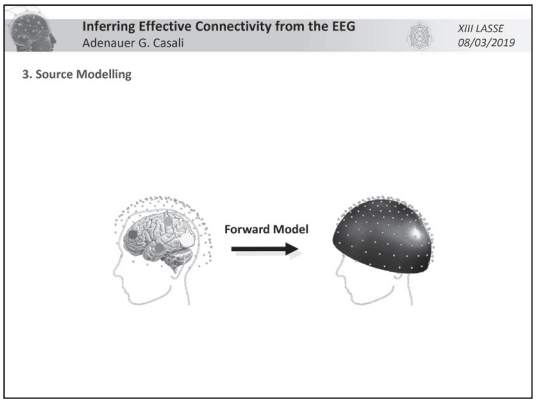
Easiness of use

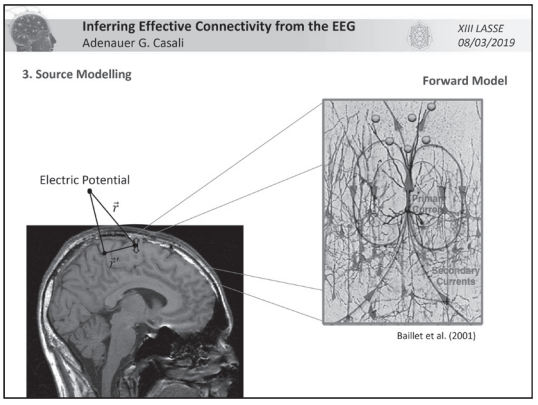
High temporal resolution


Inferring Effective Connectivity from the EEG
Adenauer G. Casali
XIII LASSE
08/03/2019
2. EEG
Influx of positive ions (EPSP)
Synapse
Depolarization
DIPOLE
Extracellular
(volume) currents
Intracellular
currents
Ions flow out of neuron


Inferring Effective Connectivity from the EEG
Adenauer G. Casali
XIII LASSE
08/03/2019
2. EEG
Amplifier EEG
EEG electrode
Scalp
Skull
Dura mater
Arachnoid
Subarachnoid space
Pia mater
Active synapse
Afferent axon
Efferent axon


Inferring Effective Connectivity from the EEG
Adenauer G. Casali
XIII LASSE
08/03/2019
3. Source Modelling
Forward Model
+
Inverse Solution
?


Inferring Effective Connectivity from the EEG
Adenauer G. Casali
XIII LASSE
08/03/2019
3. Source Modelling
Forward Model



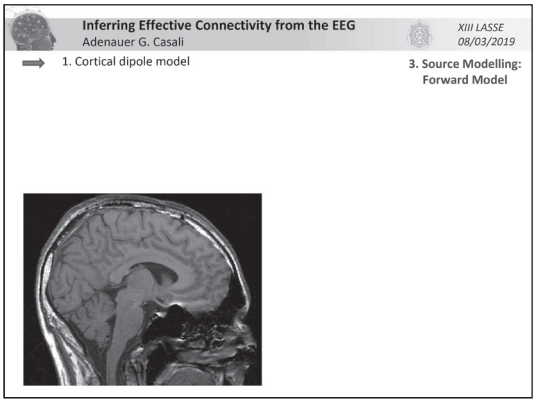
Inferring Effective Connectivity from the EEG
Adenauer G. Casali

XIII LASSE
08/03/2019

3. Source Modelling

Forward Model

Baillet et al. (2001)

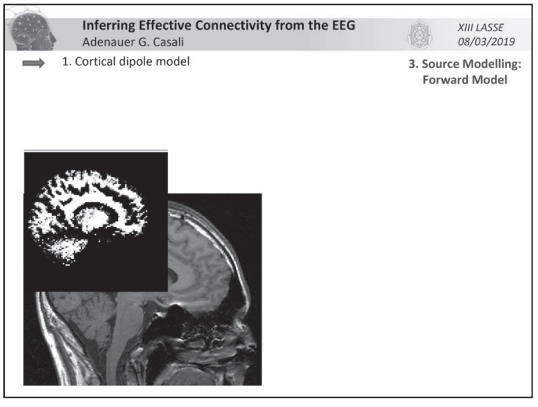


Inferring Effective Connectivity from the EEG
Adenauer G. Casali

XIII LASSE
08/03/2019

1. Cortical dipole model

3. Source Modelling:
Forward Model



Inferring Effective Connectivity from the EEG
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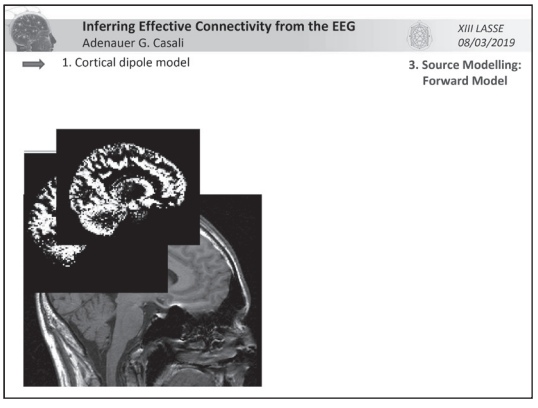
XIII LASSE
08/03/2019

1. Cortical dipole model

3. Source Modelling:
Forward Model

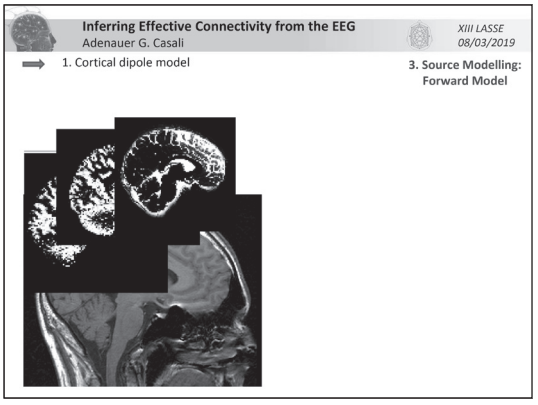


Inferring Effective Connectivity from the EEG
Adenauer G. Casali

XIII LASSE
08/03/2019

1. Cortical dipole model

3. Source Modelling:
Forward Model



Inferring Effective Connectivity from the EEG
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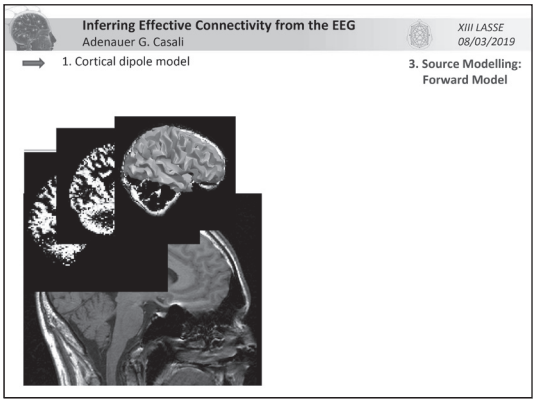
XIII LASSE
08/03/2019

1. Cortical dipole model

3. Source Modelling: Forward Model



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Adenauer G. Casali

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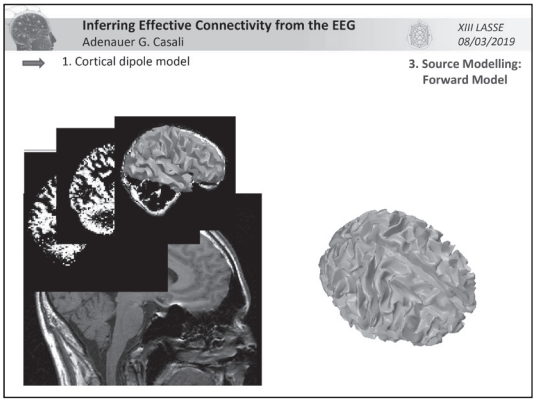
1. Cortical dipole model

3. Source Modelling: Forward Model



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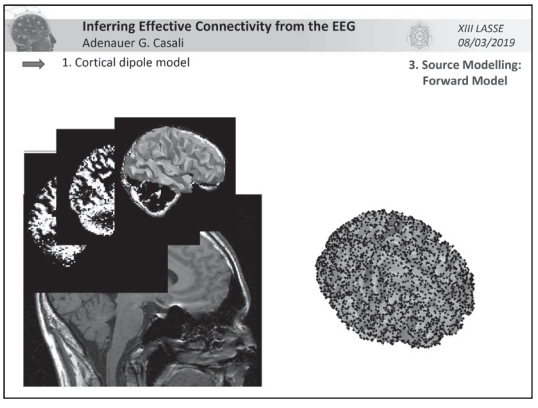

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1. Cortical dipole model

3. Source Modelling: Forward Model

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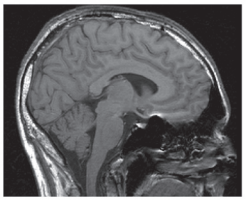
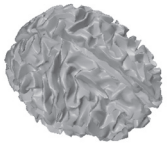
1. Cortical dipole model
2. Head tissue conductivities

3. Source Modelling: Forward Model





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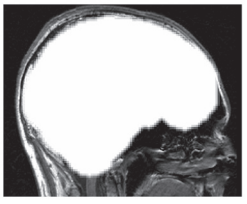

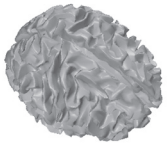

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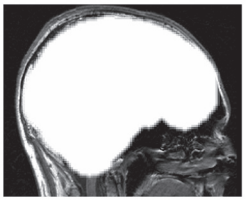
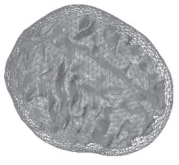
3. Source Modelling: Forward Model

Skull





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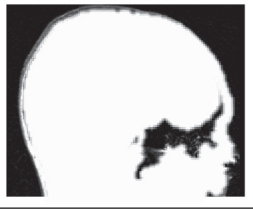

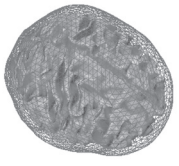

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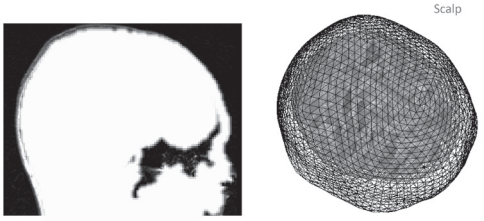
3. Source Modelling: Forward Model

- 1. Cortical dipole model
- 2. Head tissue conductivities



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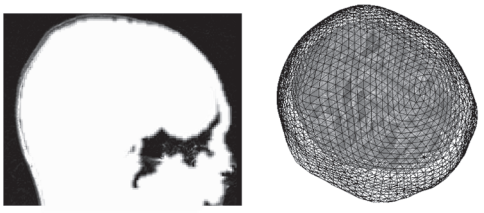
3. Source Modelling: Forward Model

- 1. Cortical dipole model
- 2. Head tissue conductivities
  - BEM: Real geometry + Isotropic and homogeneous conductivities



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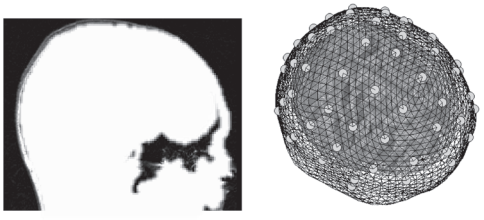
3. Source Modelling: Forward Model

- 1. Cortical dipole model
- 2. Head tissue conductivities
  - BEM: Real geometry + Isotropic and homogeneous conductivities
- 3. Electrodes positions



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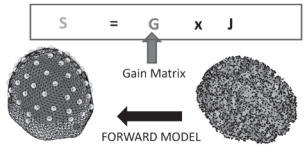
3. Source Modelling

$$S = G \times J$$



$p(S \mid J)$ = Probability of S given J

3. Source Modelling

S = G x J

$$p(\mathbf{S}\,|\mathbf{J}) = N\,(\mu = \mathbf{G} \times \mathbf{J},\ \Sigma_\varepsilon)$$

Normal distribution

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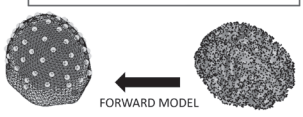
3. Source Modelling

S = G x J



$$p(\mathbf{S}\,|\mathbf{J}) = N\,(\mu = \mathbf{G} \times \mathbf{J},\ \Sigma_\varepsilon)$$

Expected value (average)

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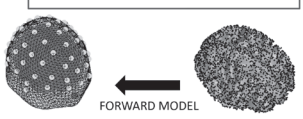
3. Source Modelling

S = G x J



$$p(\mathbf{S}\,|\mathbf{J}) = N\,(\mu = \mathbf{G} \times \mathbf{J},\ \Sigma_\varepsilon)$$

Noise Covariance

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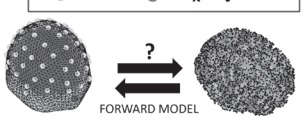
3. Source Modelling

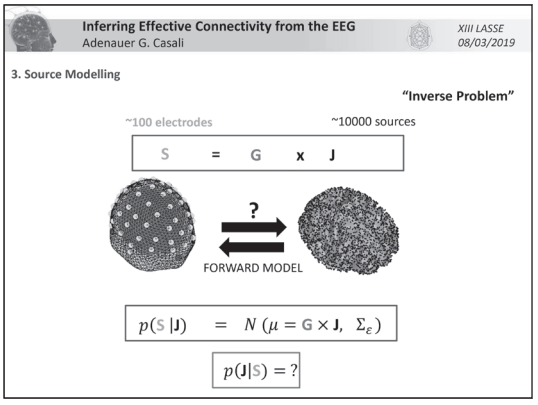
"Inverse Problem"

~100 electrodes ~10000 sources

S = G x J



$$p(\mathbf{S}\,|\mathbf{J}) = N\,(\mu = \mathbf{G} \times \mathbf{J},\ \Sigma_\varepsilon)$$


Inferring Effective Connectivity from the EEG
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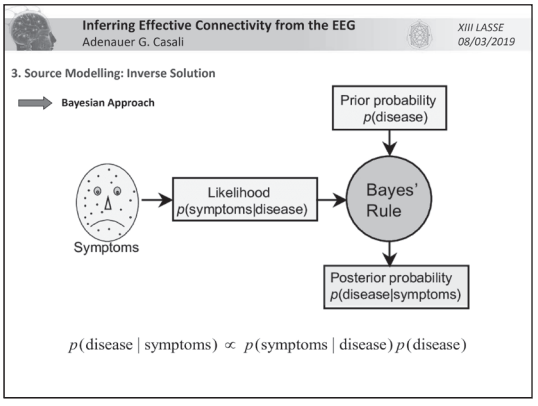
3. Source Modelling
"Inverse Problem"
~100 electrodes
~10000 sources
S = G x J
?
FORWARD MODEL
$p(S|J) = N(\mu = G \times J, \Sigma_\varepsilon)$
$p(J|S) = ?$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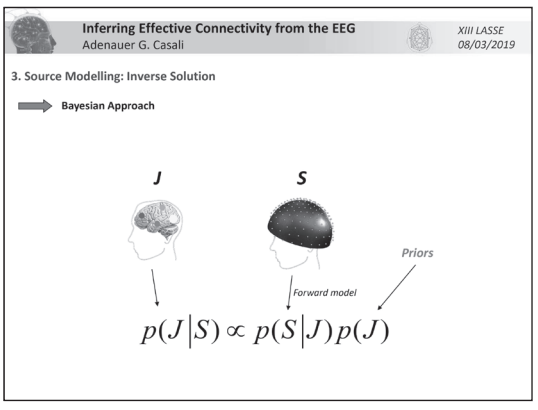
3. Source Modelling: Inverse Solution
Bayesian Approach
Prior probability
p(disease)
Likelihood
p(symptoms|disease)
Bayes' Rule
Symptoms
Posterior probability
p(disease|symptoms)
$p(\text{disease} \mid \text{symptoms}) \propto p(\text{symptoms} \mid \text{disease})\, p(\text{disease})$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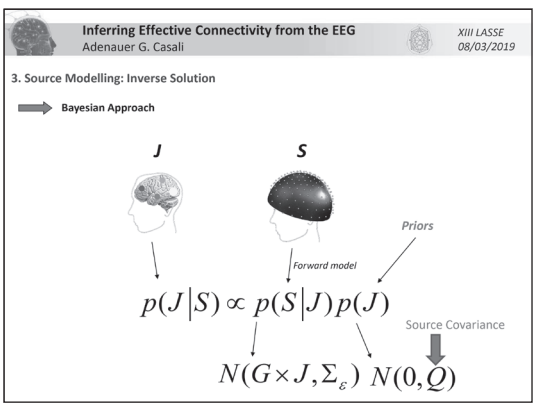
3. Source Modelling: Inverse Solution
Bayesian Approach
J
S
Priors
Forward model
$p(J|S) \propto p(S|J)p(J)$


Inferring Effective Connectivity from the EEG
Adenauer G. Casali
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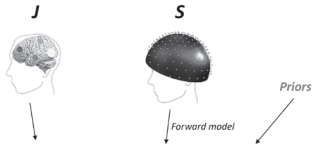
3. Source Modelling: Inverse Solution
Bayesian Approach
J
S
Priors
Forward model
$p(J|S) \propto p(S|J)p(J)$
Source Covariance
$N(G \times J, \Sigma_\varepsilon)$ $N(0, Q)$

3. Source Modelling: Inverse Solution

Bayesian Approach

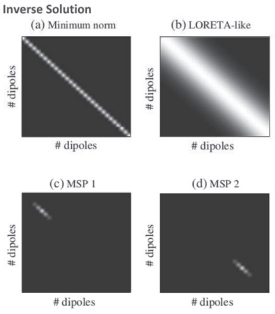


$$p(J \mid S) \propto N(G \times J, \Sigma_\varepsilon) N(0, Q)$$

Maximum posterior probability (Gaussian assumptions):

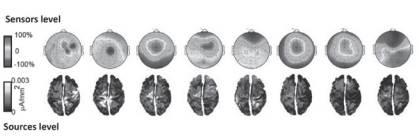
$$J = QG^T(\Sigma_\varepsilon + GQG^T)S$$

3. Source Modelling: Inverse Solution



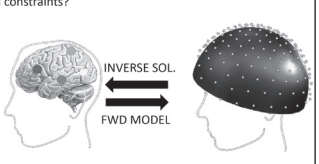
Source: Lopes et al., (2014)

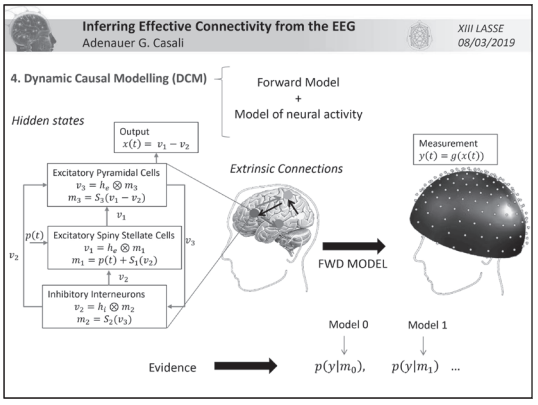
3. Source Modelling: Inverse Solution



3. Source Modelling

Inverse Solution: what about neural constraints?

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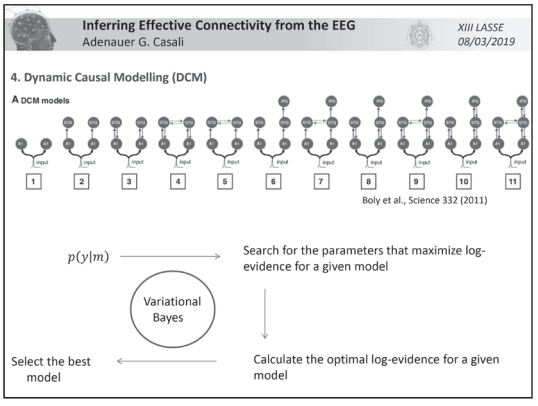
4. Dynamic Causal Modelling (DCM)
Forward Model
+
Model of neural activity
Hidden states
Output
$x(t) = v_1 - v_2$
Measurement
$y(t) = g(x(t))$
Extrinsic Connections
Excitatory Pyramidal Cells
$v_3 = h_e \otimes m_3$
$m_3 = S_2(v_1 - v_2)$
v_1
$p(t)$
Excitatory Spiny Stellate Cells
$v_1 = h_e \otimes m_1$
$m_1 = p(t) + S_1(v_2)$
v_3
v_2
v_2
Inhibitory Interneurons
$v_2 = h_i \otimes m_2$
$m_2 = S_2(v_3)$
FWD MODEL
Model 0
Model 1
Evidence
$p(y|m_0)$,
$p(y|m_1)$ ...


Inferring Effective Connectivity from the EEG
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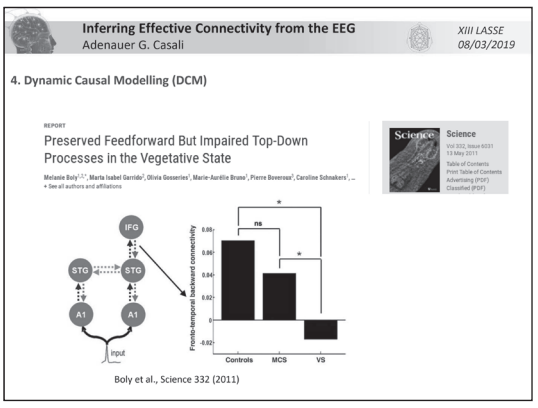
4. Dynamic Causal Modelling (DCM)
A DCM models
Boly et al., Science 332 (2011)
$p(y|m)$
Search for the parameters that maximize log-evidence for a given model
Variational Bayes
Calculate the optimal log-evidence for a given model
Select the best model


Inferring Effective Connectivity from the EEG
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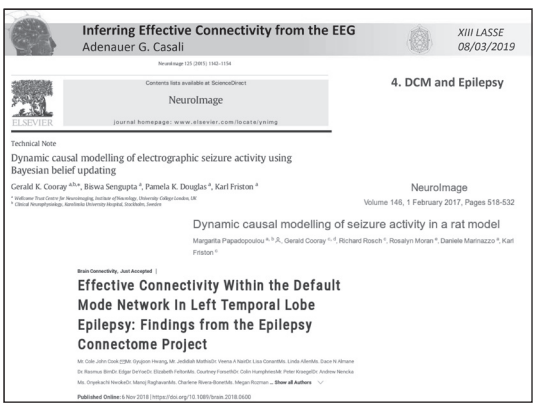
4. Dynamic Causal Modelling (DCM)
REPORT
Preserved Feedforward But Impaired Top-Down Processes in the Vegetative State
Science
IFG
STG
STG
A1
A1
Input
Fronto-temporal backward connectivity
ns
*
*
Controls
MCS
VS
Boly et al., Science 332 (2011)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4. DCM and Epilepsy
NeuroImage
Technical Note
Dynamic causal modelling of electrographic seizure activity using Bayesian belief updating
Gerald K. Cooray, Biswa Sengupta, Pamela K. Douglas, Karl Friston
NeuroImage
Volume 146, 1 February 2017, Pages 518-532
Dynamic causal modelling of seizure activity in a rat model
Effective Connectivity Within the Default Mode Network In Left Temporal Lobe Epilepsy: Findings from the Epilepsy Connectome Project

Summary

What are the different meanings of the word "connectivity" in neuroscience?

Effective connectivity: why is EEG a good method?

Source modelling: what is it and why do we need it?

Dynamic causal modelling: what is it and what can it do?

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Software

SPM

By members & collaborators of the Wellcome Centre for Human Neuroimaging

Introduction | Software | Documentation | Courses | Email list | Data | Extensions

SPM12

https://www.fil.ion.ucl.ac.uk/spm/software/spm12/

MNE

MEG + EEG ANALYSIS & VISUALIZATION

Open-source Python software for exploring, visualizing, and analyzing human neurophysiological data: MEG, EEG, sEEG, ECoG, and more.

https://martinos.org/mne/stable/index.html

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Thank you!

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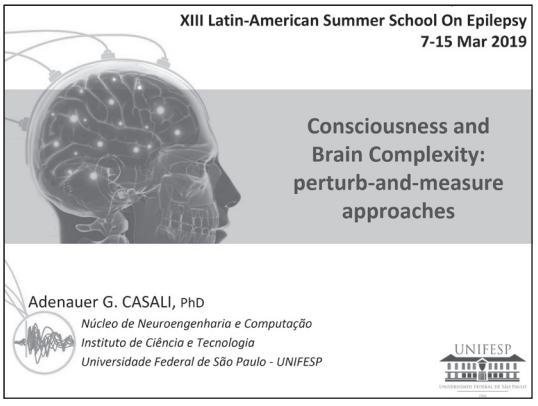
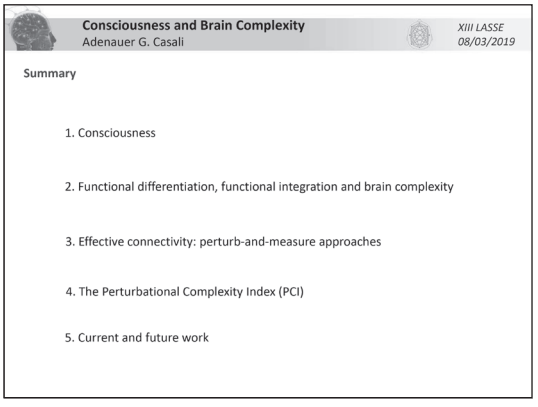
Edson Amaro (Brazil)

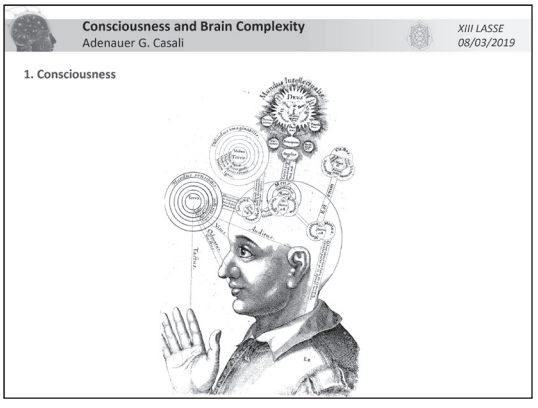
QUANTITATIVE METHODS IN NEUROIMAGING: BASIS FOR CONNECTIVITY MEASURES

Adenauer Casali (Brazil)

CONSCIOUSNESS AND BRAIN COMPLEXITY: PERTURB-AND-MEASURE APPROACHES







Consciousness and Brain Complexity
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08/03/2019

1. Consciousness

MENU nature human behaviour

Comment | Published: 04 February 2019

Opportunities and challenges for a maturing science of consciousness

Scientific research on consciousness is critical to multiple scientific, clinical, and ethical issues. The growth of the field could also be beneficial to several areas including neurology and mental health research. To achieve this goal, we need to set funding priorities carefully and address problems such as job creation and potential media misrepresentation.

Matthias Michel, Diane Beck, Ned Block, Hal Blumenfeld, Richard Brown, David Carmel, Marisa Carrasco, Mazviita Chirimuuta, Marvin Chun, Axel Cleeremans, Stanislas Dehaene, Stephen M. Fleming, Chris Frith, Patrick Haggard, Biyu J. He, Cecilia Heyes, Melvyn A. Goodale, Liz Irvine, Mitsuo Kawato, Robert Kentridge, Jean-Remi King, Robert T. Knight, Sid Kouider, Victor Lamme, Dominique Lamy, Hakwan Lau, Steven Laureys, Joseph LeDoux, Ying-Tung Lin, Kayuet Liu, Stephen L. Macknik, Susana Martinez-Conde, George A. Mashour, Lucia Melloni, Lisa Miracchi, Myrto Mylopoulos, Lionel Naccache, Adrian M. Owen, Richard E. Passingham, Luiz Pessoa, Megan A. K. Peters, Dobromir Rahnev, Tony Ro, David Rosenthal, Yuka Sasaki, Claire Sergent, Guillermo Solovey, Nicholas D. Schiff, Anil Seth, Catherine Tallon-Baudry, Marco Tamietto, Frank Tong, Simon van Gaal, Alexandra Vlassova, Takeo Watanabe, Josh Weisberg, Karen Yan and Masatoshi Yoshida

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1. Consciousness

VIDEO

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1. Consciousness

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1. Consciousness

The Naturalistic hypothesis

Consciousness is a natural phenomenom:
We can explain consciousness in terms of basic natural laws.

However...

... Consciousness is not an ordinary natural phenomenom...

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1. Consciousness

Dolores **having** subjective experience

Dolores lacking subjective experience

Consciousness does not supervene logically on the physical!

David Chalmers
NYU

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1. Consciousness

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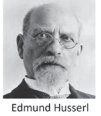
1. Consciousness

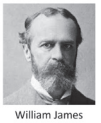
Giulio Tononi

Experience is structured and specific



Edmund Husserl

Experience is unitary



William James

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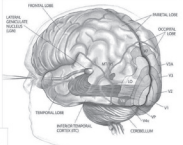
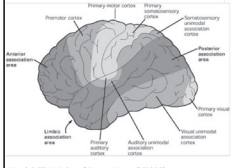
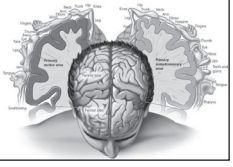
2. Functional Differentiation and Functional Integration







Kandel, "Principles of Neuroscience" (2000)

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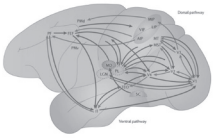
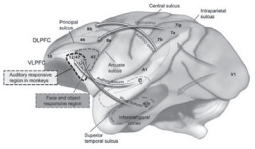
2. Functional Differentiation and Functional Integration



Gilbert and Li, *Nature* (2013)



Romanski, *Cereb. Cortex* (2007)

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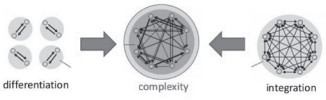
2. Functional Differentiation and Functional Integration

The Complexity Hypothesis

Consciousness requires an optimal balance between functional integration and functional differentiation in thalamocortical networks



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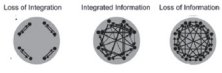
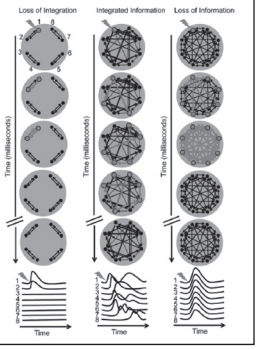
3. Perturb-and-measure approaches





Sarasso et al. (2014)

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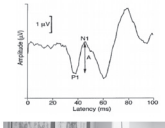

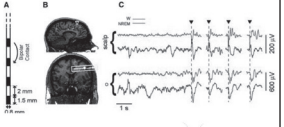

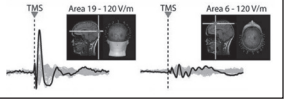

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3. Perturb-and-measure approaches

Transcranial Magnetic Stimulation (TMS) +
Navigated Brain Stimulation (NBS) +
high-density Electroencephalography (hd-EEG)

VIDEO

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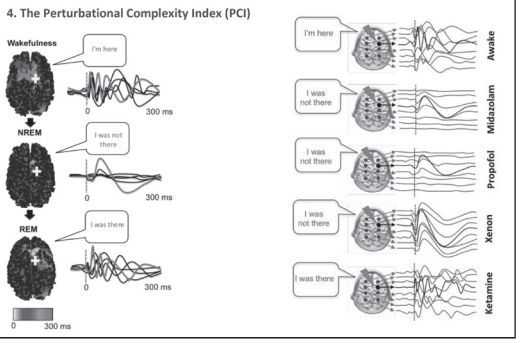
4. The Perturbational Complexity Index (PCI)



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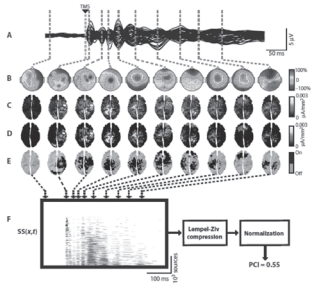
4. The Perturbational Complexity Index (PCI)

1) TMS/EEG evoked potential
2) Source Modelling
3) Statistics
4) Binarization
5) Complexity metric



Casali et al. (2013)

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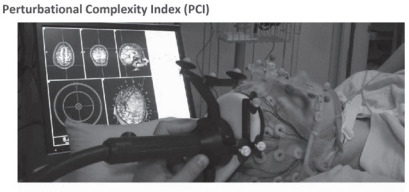

VIDEO

Casali et al. (2013)

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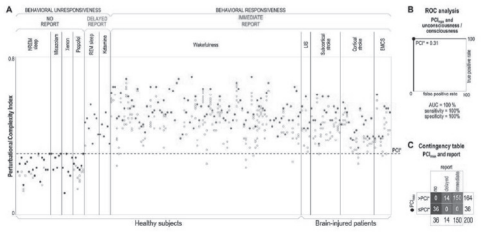
4. The Perturbational Complexity Index (PCI)

Research Article

Annals of NEUROLOGY — An Official Journal of the American Neurological Association and the Child Neurology Society

Stratification of unresponsive patients by an independently validated index of brain complexity



Casarotto et al. (2016)

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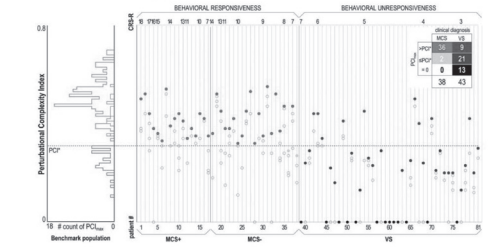

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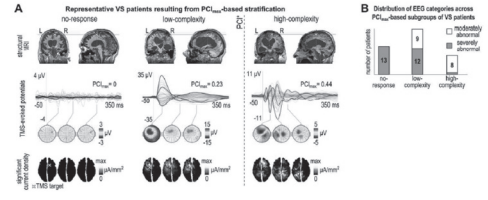

Casarotto et al. (2016)

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5. Current and future work

- Can we use PCI for the bedside assessment of consciousness?
- **Can we use PCI to reveal the mechanisms of brain complexity?**
- **How can these studies help us understand the nature of consciousness?**

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5. Current and future work

1) TMS/EEG evoked potential

2) Source Modelling

3) Statistics

4) Binarization

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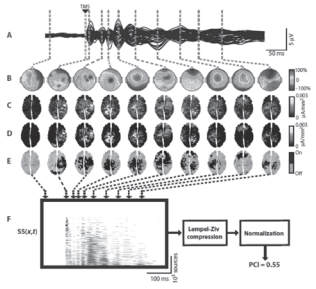

Casali et al. (2013)

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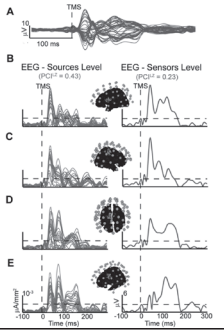

Comolatti et al. (2019)

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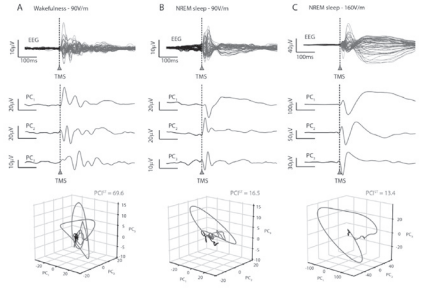

Comolatti et al. (2019)

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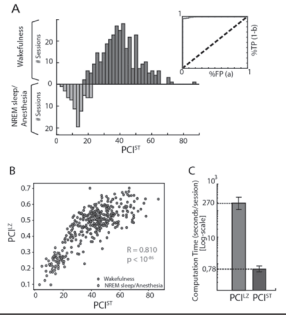

Comolatti et al. (2019)

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Can we use PCI for the bedside assessment of consciousness?

Can we use PCI to reveal the mechanisms of brain complexity?

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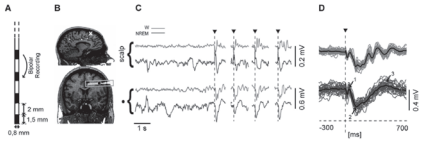
5. Current and future work

Bistability breaks-off deterministic responses to intracortical stimulation during non-REM sleep

NeuroImage
Volume 112, 15 May 2015, Pages 105–113



Pigorini et al. (2015)

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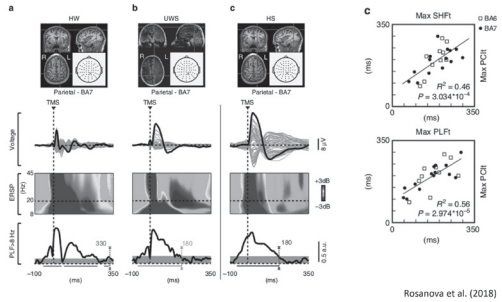
5. Current and future work



Rosanova et al. (2018)

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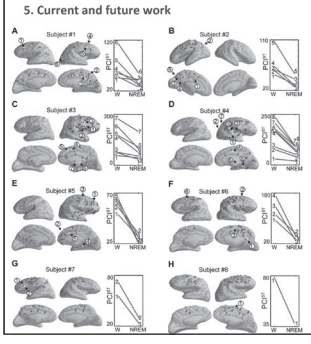
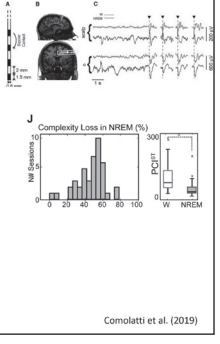
5. Current and future work

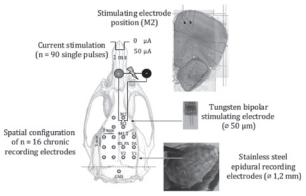




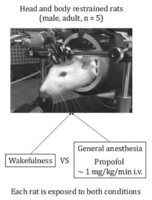
Comolatti et al. (2019)

5. Current and future work

EPIDURAL EEG RECORDING & CURRENT STIMULATION



EXPERIMENTAL CONDITIONS



Arena et al., Society for Neuroscience 2018

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Can we use PCI for the bedside assessment of consciousness?

Can we use PCI to reveal the mechanisms of brain complexity?

➡ **How can these studies help us understand the nature of consciousness?**

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Matteo Fecchio
Andrea Pigorini
Simone Sarasso

THE UNIVERSITY of WISCONSIN MADISON
Giulio Tononi
Melanie Boly

Fabio Ferrarelli

Thank You!

FAPESP
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2017/03678-9
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Adriano Tort (Brazil)

THETA-GAMMA COUPLING IN THE HIPPOCAMPUS

Jaime Carrizosa (Colombia)

EPILEPSY REVEALED: THE HISTORY OF EPILEPSY THROUGH PAINTINGS

Epilepsy revealed: the history of epilepsy through paintings and sculpture

Jaime Carrizosa Moog

University of Antioquia
Child and Adolescent Neurology Service
Pediatric Department

Medellín, Colombia

Hammurabi, VI king of Babylon from 1792 BC to 1750 BC
Hammurabi's Code was one of the first written codes of law in history.

Seizure 21 (2012) 595–596

Contents lists available at SciVerse ScienceDirect

Seizure

journal homepage: www.elsevier.com/locate/yseiz

The epileptic seizure and the myth of Hyakinthos

Michael W. Mann

Hôpital St Anne, Department of Neurosurgery (Pr FX Roux), 1 rue Cabanis, F-75014 Paris, France

Zephyr or Boreas and Hyacinth on an attic vase. Art Museum, Boston

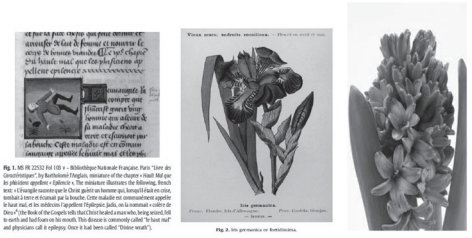
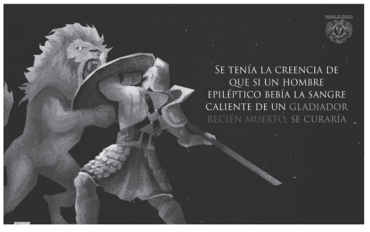


Fig. 1. MS FR 22532 Fol 11B v – Bibliothèque Nationale Française, Paris "Livre des Cirurgiens". by Bartholomé Englais, miniature of the chapter « Haut Mal que les phisiciens appellent « Epilencie ». The miniature illustrates the following, French text: « L'Evangile nacontie que le Christ guérit un homme qui, lorsqu'il était en crise, tombait à terre et écumait par la bouche. Cette maladie est communément appelée le haut mal, et les médecins l'appellent l'épilepsie. Jadis, on la nommait « colère de Dieu »" (the Book of the Gospels tells that Christ healed a man who, being seized, fell to earth and had foam on his mouth. This disease is commonly called "le haut mal" and physicians call it epilepsy. Once it had been called "Divine wrath").

Fig. 2. Iris germanica or Bartholomæa.



Mckeown J.C. Gabinete de curiosidades romanas: Relatos extraños y hechos sorprendentes (Tiempo de Historia). Ed Critica. 2011.P 336

Mosaic of Gladiators in the Galleria Borghese Rome Italy. Ancient, exhibits.

Epilepsia, 41(7):913–917, 2000
Lippincott Williams & Wilkins, Inc., Baltimore

Special Article

History of Epilepsy

The Epilepsy of Emperor Michael IV, Paphlagon (1034–1041 A.D.): Accounts of Byzantine Historians and Physicians

*J. Lascaratos and †P. V. Zis

*Departments of *History of Medicine and †Neurology, Medical School, National Athens University, Athens, Greece*

Emperor Romanos III Argyros (968-1034 AD) + Empress Zoe the Porphyrogenitus (978 – 1050 AD)

Eunuch John Orphanotrophus

Emperor Michael IV, Paphlagon (1010 – 1041 AD)

Numismatic Museum of Athens. Mosaics at the Agia Sophia, Istanbul.
The murder of Romanos III Argyros in a bath, from the *Chronicle* of John Skylitzes.

FIG. 1. Emperor Michael IV, Paphlagon, as presented on a gold coin during his reign (Numismatic Museum of Athens).

Epilepsia, **40**(7):1041–1046, 1999
Lippincott Williams & Wilkins, Inc., Philadelphia

Historical Article

Epilepsy and Its Treatment in the Ancient Cultures of America

Jan G. R. Elferink

Department of Molecular Cell Biology, University of Leiden, Leiden, The Netherlands

FIG. 1. Illustration from the work of Poma de Ayala (14), representing the wife of Inca Capac Yupanqui during an epileptic seizure.

TABLE 1. *Indigenous names for epilepsy*

Inca names	Description	Reference
Aya huayra	Wind (air) of the dead	5
Chayapuk oncuy		6
Huanuy oncuy	Disease of the dead	12
Huani keshia	Disease of the dead	12
Llaqui oncuy	Disease of sadness	12
Sonko nanay	Disease of the heart	12
Ttucu	Owl, night bird; attack	33
Urmachiscan	'He is thrown on the ground'	10
Aztec names		
Comic aquilizitli		13
Cuecuechmiqlizitli		13
Tlacolmimiquilizitli	Disease due to love and desire	21
Tlacolmiquizitli	Disease due to love and desire	21
Tlayouallotl tepam momana		13
Yolcotlauallizitli		13
Yollo mimiquilizitli	Disease of the heart	13
Yolpatzmiquilizitli		13

Fig. 10. *Skull trepanation scene of the pre-Inca period.* Painted in 1997. Technique: oil/canvas. Location: Lima Institute of Neurosciences. Artist: Juan Bravo Vizcarra [7].

I.D. Ladino et al. / Epilepsy & Behavior 29 (2013) 82–89

Fig. 9. *Trepanning in ancient Peru.* Painted in 1959. Technique: oil painting on masonite. From Parke, Davis Great Moments in Medicine. Collection: Michigan Medical School. Artist: Robert Thom [30].

L.D. Ladino et al. / Epilepsy & Behavior 29 (2013) 82–89

L.D. Ladino et al. / Epilepsy & Behavior 57 (2016) 255–264

Fig. 8. *"Inca skull surgery in Machu Picchu"*, mural by Alton Stanley Tobey. At the National Museum of Natural History, Smithsonian Institution, Washington DC.

Tumi: *Peruvian ceremonial knife of Chimu. Moche and Lambayeque cultures*

Ñaylamp: after founding the Lambayeque culture, he grew wings and flew into the sky.
Tumi use: Trepanations, ceremonial rituals, prisoners´cutthrouts

Associated Press 2006-11-21. Retrieved 2008-11-24.
Scott, John F. (1999). Latin American Art: Ancient to Modern. Gainesville: University Press of Florida. p. 128.

Epilepsy & Behavior 57 (2016) 60–68

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Review

Tlazolteotl, the Aztec goddess of epilepsy

Lady Diana Ladino [a], José Francisco Téllez-Zenteno [b,*]

Fig. 2. Borbon codex. The goddess squats in the birth-giving position, wearing the conical Huastec hat with Aztec symbols. Her face is painted yellow, the usual sign for the color red, decorated with crescent moons [26]. This drawing shows Tlazolteotl conceiving a child. She births a child who wears a headdress, earrings, and necklace just like her mother. While embodying this cycle of life, Tlazolteotl wears the flayed skin of the sacrificial victim (the dimpled skin always signifies this, but it is very obvious here with the extra hands hanging below), a symbol of death feeding life [23].

Between all the Aztec deities, Tlazolteotl played an important role. She was also called *Ixcuina* or *Tlaelquani*. Her name is derived from the *Nahuatl* word for garbage: *tlazolli*, which was used to connote filth [5]. The second part: *teotl*, signifies deity. According to Mesoamerican mythology, Tlazolteotl was the mother of *Centeotl*, the maize god, and of *Xochiquetzal*, goddess of fertility. As the goddess of fertility and sexuality, she was often depicted giving birth. Therefore, this divinity has

Tlazolteotl was the goddess of medicines, herbal remedies, fortune-tellers, surgeons, blood-letters, midwives, clairvoyants, and other healers [6]. All these people met once a year not only to perform a cele-

According to Aztecs, *yolpatzmiquilizti*, an epileptic fit, was ultimately caused by the rain god, but the proximate cause could be either possession by one of the god's helpers or a rapid accumulation of phlegm in the chest. States of health were related to equilibrium. The

generalized tonic–clonic, myoclonic, and auras. "*Huapahualizzti*" were epileptic disorders characterized by stillness and convulsions followed by stillness. They describe it as "the fit left the patient with a death body" (grand mal) and "*Hixcayotl*" that were epileptic episodes characterized exclusively by tremor (myoclonic seizures) [7].

the term "Tlazolli" (dung) means dirt, both material (physical) and moral (ethically). Seizures and epilepsy have historically been considered as a disease of impurities, and the goddess has the ability to clean the dirt/cure the disease [22].

Fig. 1. Famous depiction of Tlazolteotl in the German epilepsy museum showing the goddess giving the disease [25]. It is the logo of the Mexican Chapter of the International League Against Epilepsy.

Fig. 3. This is a depiction of Tlazolteotl from the codex Borgia manuscript, sheet 55. In this depiction, the goddess is wearing the crescent-shaped nose ring, which refers clearly to the moon. Tlazolteotl was the lunar deity par excellence. She has small moons as decoration on her garments. According to the Aztecs, "the moon is the first death, disappears from the sky for three nights, but then regenerated, returns, comes back to life, and therefore ensures the dead a new way of existence" [30].

Tlazolteotl represented sexual impurity and sinful behavior. When Tlazolteotl was a young woman, she was a carefree temptress. In her second form, she was the destructive goddess of gambling and uncertainty. In her middle age, she was the great goddess able to absorb human sin. In her final manifestation, she was a destructive and terrifying hag praying upon youths. Tlazolteotl was thought to provoke both lust and lustful behavior, but she also could grant absolution to those who had sinned. She became best known for her capacity to cleanse such sins during confessionals conducted by her priest. Although she could in one form inspire debauched behavior, she could also forgive sinners and remove corruption from the world [18].

Fig. 4. Among the major characteristics of the deity, it is recognized the semicircular nose ring, the presence of moons in her dress by alluding to the night, and the presence of the skull in the neck, in connection with death. The goddess is accompanied by a bird that is usually eating trash (crow) and has also a black mouth [27].

Fig. 5. a. Photograph by Douglas Miller/Topical Press Agency/Getty Images (http://sikn.tsiuk.tumblr.com/post/10134467819). The Aztec goddess Tlazolteotl was a complex deity, known in one aspect for luring people into bad behavior and in another for absolving them of their sins. This figurine at the Tate Gallery in London shows Tlazolteotl during childbirth in her mother goddess aspect. b. Tlazolteotl sculpture: huaxina, statuette en wernerite. Huastec, 900–1521 AD (Manzanilla, 1985). Robert Woods Bliss Collection, Washington. Here, the deity is giving birth and has her mouth open and her bare chest [27]. The sculpture has been described as a masterpiece, highly sophisticated in design and execution (photo published in 1907, in Journal de la Société des Américanistes) [21].

Fig. 6. Sculpture at the National Museum of Anthropology, Mexico City. The style of the sculpture corresponds to Tlazolteotl cultures developed by the subregion of the south-central state of Veracruz. The origin is Huastec. Notice the black paint that frames the mouth, one of the identifying attributes of the goddess known as *Motenokspintica* [19].

Fig. 7. The bell of Giovanni of Modena, 1410, Bologna, San Petronio, Chapel Bolognetti. The goddess is represented as a deity inspired by the devil [27].

Fig. 8. My birth by Frida Kahlo. In this painting, the head of Frida is leaving from the mother's womb. There is a puddle of blood under the mother's body, which might be a hint of the artist's own experience with a recent miscarriage. A sheet covers the mother's face. Above the birth bed, a picture of the weeping "Virgin of sorrows" hangs. The Virgin looks on in tears with sorrow and sympathy.

L.D. Ladino, J.F. Téllez-Zenteno / Epilepsy & Behavior 57 (2016) 60–68 67

Fig. 9. Mural: "The History of medicine in Mexico" by Diego Rivera, at the Hospital de la Raza in Mexico City. In the middle of the painting, there is a representation of Tlazolteotl.

Fig. 10. Tlazolteotl the Aztec goddess of epilepsy. Modern representation by the artist Eduardo Urbano Merino (www.eduardourbano.com).

7 Sociocultural History of Epilepsy

Peter Wolf

Figure 7-1. Jesus Christ cures an epileptic boy. Attributed to the "Third Painter of the Glajor Gospels", Armenia, 1st quarter of the 14th century. UCLA Digital Library Program. The chain around the boy's waist seems to refer to the risk of falling into fire and water. In the best-known image of the scene, Raffaello Sanzio's (1483–1520) Transfiguration of 1520 (Fig. 101-1), the body position seems to indicate that the painter has seen tonic postural seizures

Figure 7-2. Peter Paul Rubens (1577–1640): Saint Ignatius of Loyola exorcising (1617). Wien, Kunsthistorisches Museum. Note the realistic depiction of generalized tonic–clonic seizures including deep cyanosis, which seems to be based upon own observations

Figure 7-4. Ex-voto from the pilgrimage of Altötting (Bavaria) depicting a child with retroversive absence. Photo by Claus Hansmann, Stockdorf

a

b

c

Figure 7-6. São José de Ribamar is a local saint with exceptional powers in the seaside port of the same name in the state of Maranhão in the North of Brazil. Numerous ex-votos dedicated to him witness of his miraculous help in illness, tempest at sea and other adversities. Ex-votos are for sale both in the church and in a nearby shop specialized in objects of devotion. Asked about the correct ex-voto for epilepsy both the sexton in the church and the staff of the shop without hesitation recommended a waxen model of a head or, in case of small children, of a whole body. (a) The saint's grotto and statue (author's photo). (b) The church dedicated to the Saint (source: wikimedia commons). (c) Waxen models for sale as ex-votos in the church (author's photo)

THE SAINTS OF EPILEPSY

by

EDWARD L. MURPHY

EPILEPSY, at least in its grand mal variety, presents so dramatic and, to the lay observer, so terrifying a spectacle that it is not strange that its victims readily resorted to supernatural aid for alleviation. Unlike so many other diseases it offers no external signs of its presence and the horrifying suddenness with which apparently healthy and normal people could be transformed into writhing convulsives must have gone a long way in suggesting that the syndrome resulted from visitations of God or from His temporary defeat by the powers of evil. As we know, the Greeks thought of the disease as a divine intervention in the life of man, although the critical voice of Hippocrates had announced that it was no more divine than any other ailment. In early Christian times, and

Medical History Vol 3 (4); 1959

Los Sirvientes de los Epilépticos

(Salveregina2005)

Esta iniciativa vio la luz el 18 de abril de 2013.
Se dirige a aquellas personas que sufren de epilepsia, sobre todo, cuando esta enfermedad es originada por prácticas mágicas.

Patrona la Virgen Santísima en distintas advocaciones marianas :
Nuestra Señora de Montserrat
Nuestra Señora de Einsiedeln
Nuestra Señora de Lourdes
Nuestra Señora de Fátima
Nuestra Señora de Torrecíudad
Nuestra Señora de la Caridad del Cobre
Nuestra Señora Auxiliadora
Nuestra Señora de Africa

Protectores (entre otros) : Los Coros Celestiales, san Marcial, san Benito, santa Escolástica, san Juan Bosco, santo Domingo Savio, san José Cafasso, san Romualdo, san Maximiliano Kolbe, santa Teresa de Avila, san Pedro de Alcántara, san Guy de Pomposa, Venerable Meinrad Eugster, san Mathurin, san Willibrordo, santa Catalina de Siena, santo Domingo de Guzmán, san Pio de Pietrelcina, san Francisco de Asís, san Francisco Javier, san Ignacio de Loyola, san José María Escrivá, Venerable Alvaro del Portillo, santa Devota, san Juan Bautista, santa Bernardita Soubirous, Beata Mirjam Abellin, santa Teresita del Niño Jesús, santa Maravillas de Jesús, san Juan de Avila, santa Genoveva Torres, san Antonio de Padúa, san Camilo de Lellis, beata Margarita Bays, san Bernardo, san Antonio el Grande, san Pablo el Ermitaño, san Macario el Grande, san Atanasio el Atónita y los santos Atónitas, santa Benedicta y santa Bibiana.

Epilepsy & Behavior 57 (2016) 255–264

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Review

Epilepsy through the ages: An artistic point of view

Lady Diana Ladino [a,b,1], Syed Rizvi [a], Jose Francisco Téllez-Zenteno [a,*]

Saint Valentine

Fig. 3. "Saint Valentine" votive painting by the Hans Georg Geigerfeld, oil painting, 1675 (artist unknown), art collection of the Franciscan monastery in Klanjec, Croatia.

Fig. 2. "Saint Valentine patron of epileptics", woodcut of 30.4 × 31.8 cm, 1470/1480 (artist unknown), National Gallery of Art, Washington DC.

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Fig. 4. "Saint Valentine", ceiling painting, 1740, Bamberg, Unterleiterbach, Germany.

Saints and ExVotos

Der Heilige Antonius
Exvoto um 1773 aus der Kapelle St. Antonius in Haidlfing
(Photographie Claus Hansmann, Stockdorf)

Saints and ExVotos

Heilung einer Besessenen
Exvoto um 1700. Majolika-Tafel aus der Wallfahrtskriche Madonna dei Bagni/Umbrien
(Photographie Claus Hansmann, Stockdorf)

Saints and ExVotos

Tafelbilder der Kirche in Krummenau aus der
Werkstatt des Johann Georg Engisch
Epilepsie Museum Kork

Saints and ExVotos

Jesus cures an epileptic
Anonymous

Medieval image of exorcism Diablo,
14th Century, Byzantine

Limbourg Brothers (Herman, Jean, Pol): The Exorcism in the Synagogue,
Les Très Riches Heures du Duc de Berry
Musée Condé, Château de Chantilly, 1412-16

"Jesus cures an epileptic demonic child", French School XVII Century
www.subastasimperio.com

Verona, Basilica di San Zeno

Traían unos hombres a una mujer endemoniada, atada con cuerdas, quien apenas vio a la Santa rompió en gritos y se revolcó por el suelo. Catalina acercándose pidió que la soltaran en nombre de Jesucristo e inmediatamente ésta quedó libre de sus tormentos.

Monastery of Saint Catherine of Sienne, Arequipa – Peru
Painter Unknown

Aquí pinté a mi padre Wilhelm Kahlo, de origen húngaro-alemán, artista-fotógrafo de profesión, de carácter generoso, inteligente y bueno, valiente porque sufrió durante sesenta años de epilepsia pero nunca se rindió trabajando y luchó contra Hitler, con adoración, Su hija Frida Kahlo".

Fig. 5. "Die Epileptiker tanzen von Molenbeeck", engraving by Hendrick Hondius the Younger (1642) after a pen-and-ink drawing by Breughel the Elder (1564), original size: 28.6 cm × 41.4 cm, at the Bibliothèque royale Albert Ier (Cabinet des Estampes), Brussels, Belgium.

Fig. 6. "*The Pilgrimage of the Epileptics to the church at Molenbeek*" also called "*The dance of St. John or St. Vitus*" by Pieter Bruegel, known as "Bruegel the Younger". Wood panel painting, original size: 29.2 cm × 62.2 cm. At the Royal Institute for the Study and Conservation of Belgium's Artistic Heritage, Brussels, Belgium.

The Transfiguration, Raphael Sanzio 1483 - 1520
Vatican Museum

The Transfiguration of Christ, Peter Paul Rubens (1577 – 1640)
Museum of Fine Arts of Nancy, France

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Review

Art and epilepsy surgery

Lady Diana Ladino [a,b], Gary Hunter [a], José Francisco Téllez-Zenteno [a,*]

[a] Division of Neurology, Departmmt of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[b] Neurology Department, College of Medicine, University of Antioquia, Medellín, Antioquia, Colombia

Image from the 14th century, a physician performs trepanation, a surgical procedure then used to treat epilepsy.

Guido da Vigevano 1280 - 1349
Reproduction of *Anathomia* (1345)

Fig. 1. *Epileptricus Sic Curabitur* ("*the way to cure an epileptic*") Sloane manuscript, collection of medical manuscripts. Miniature painted at the end of the 12th century. Collection: British Museum, London. Artist: unknown [16].

Fig. 2. Epilepsy treatment from the surgical atlas *Cerrahiyetül Hanîye* (Imperial surgery). Drawn in the 15th century (1465). Collection: Ataturk Institute for Modern Turkish History, Istanbul, Turkey. Artist: Sefereddin Sabuncuoğlu [17].

TREPANATIONS

Fig. 3. *The cure of folly* or *The extraction of the stone of madness*. Painted around 1485. Technique: oil on board. Collection: El Prado Museum, Madrid, Spain. Artist: Hieronymus van Aeken [22].

TREPANATIONS

Fig. 4. *The surgeon*. Painted in 1555. Technique: oil painting. Height: 100 cm, width: 141 cm. Collection: El Prado Museum, Madrid, Spain. Artist: Jan Sanders Van Hemessen [11].

TREPANATIONS

Fig. 5. *Cutting out the stone of madness* or *An operation on the head*. Painted in 1568. Collection: Museum d'Isbel Sandelin, Saint Omer, France. Artist: Pieter Brueghel, the Elder. Apparently the original is lost, and there are multiple copies [23].

Fig. 7. *Surgical operation on a man's head* or *Fool's stone*. Painted between 1680 and 1690. Technique: oil/canvas. Collection: El Prado Museum, Madrid, Spain. Artist: David Teniers the Younger [26].

Cutting the stoner, Pieter Jan Quast (1605 – 1647)
Kunstmuseum Sankt Gallen, Switzerland

Hieronimus van Aken, "El Bosco" (1450-1516)
Prado Museum, Madrid

A surgeon extracting "pierres de tête". Oil painting after Nicolaes Weydmans (1570 - 1642) Wellcome Collection gallery

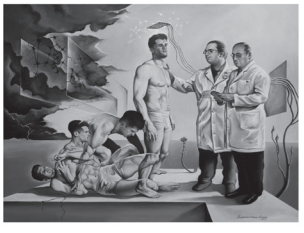

Fig. 11. *Epilepsy, leaving the nightmare behind.* Painted in 2013. Technique: oil/canvas. Measurements: 100 × 120 cm. Location: Royal University Hospital, Saskatoon, Saskatchewan, Canada. Artist: Eduardo Urbano Merino.

Fig. 12. "Wired", 70 × 90 alkyd and oil on canvas 2011, by Iris Hauser, Saskatoon, Saskatchewan, Canada. Reprinted with permission from the artist.

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Review

The Montreal procedure: The legacy of the great Wilder Penfield

Lady Diana Ladino [a,*], Syed Rizvi [b], José Francisco Téllez-Zenteno [b]

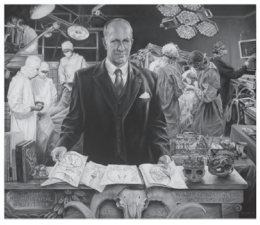
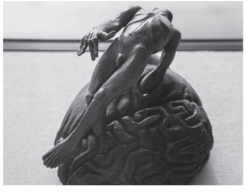


Fig. 10. The portrait by Iris Hauser. On the left is the Montreal Neurological Institute theater at Wilder Penfield's era. On the right is the current Royal University Hospital operating room at Saskatoon, Saskatchewan, Canada. The figure represents the evolution of the Montreal procedure from the past to the present, not only in Canada, but also throughout the entire world.

Pierre Andre Brouillet (1857-1914), " Une leçon clinique à la Salpêtrière"
Paris Descartes University, Paris

Tony Robert Fleury (1837-1912), "Philippe Pinel à la Salpêtrière"
British Museum

Bohumil Kubista (1884-1918), "Epileptic Woman"
Moravian Gallery in Brno



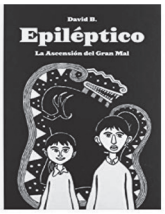
Fallsucht von Bodo Wentz
Epilepsy Museum, Kork Germany

The ceramic depicts two essential moments of the disease epilepsy, firstly the ***medical*** aspect: the brain as the source of the disease, and the "seizure" of the person suffering from epilepsy as the impressive symptom of the disease.
Secondly it portrays the ***psycho-social*** aspect: the chronic disease "epilepsy" leading to exclusion, social isolation, desolation, retreating ("into one's shell").

Fig. 9. Poor Miss Finch by unknown artist, 1872, from the book Poor Miss Finch.

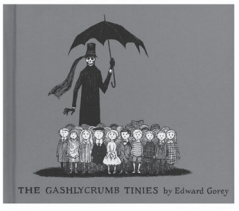
Willkie Collins, 1824 - 1889

Fig. 10. *The Red Curtain or Hommage to Vincent*, Anonymous, approx. 1965, German Epilepsy Museum, Kork, Germany.



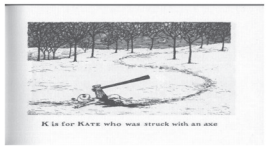
Pierre-François Beauchard
Ediciones Sinsentido, 1996

"¿Se puede vivir así? No tenemos otro remedio. La enfermedad se ha instalado en casa sin pedirnos permiso. "



U is for Una who slipped down a drain

S is for Susan who perished of fits



K is for Kate who was struck with an axe

Z is for Zillah who drank too much gin

CONCLUSIONS

Plastic art of epilepsy through history reveal the mythologic religious or supranatural explanations of the disorder according to the prevailing sociocultural knowledge.

Paintings and sculptures demonstrate the suffering of persons with epilepsy and offer different ways of healing that could be complimentary to medical treatments.

The stigmatizing or de-stigmatizing effect of epilepsy through plastic art has to be determined.

Matteo Mancini (UK)

THE DIFFUSION MRI-BASED CONNECTOME: PROMISES AND PITFALLS

UCL

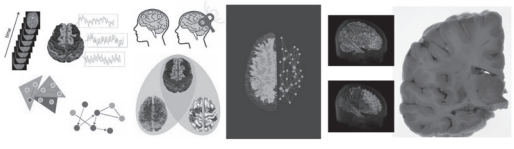
The diffusion MRI-based connectome: promises and pitfalls

Dr. Matteo Mancini
University College London

Outline

- About me;
- Network models and graph theory;
- The brain as a network;
- Diffusion MRI;
- Defining nodes;
- Defining edges;
- Network properties and graph measures;
- Current issues and pitfalls;
- Translating into clinical applications;
- Take-home message.

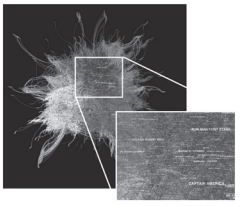
About me



PhD (Rome -> Utrecht -> Brighton) | Postdoc (UCL)

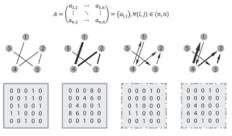
Networks and graphs

- Network science is a relatively new discipline that studies complex systems focusing on their actors and relational linkages;
- Applications:
  - Internet;
  - Social networks;
  - Marketing;
  - Epidemiology;
  - Biology;
  - Neuroscience;
  - …and beyond!



Networks and graphs

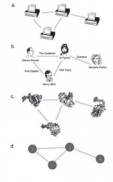
- Graph theory represents the mathematical formalism used in network science;
- A graph is a set of nodes and edges, where the nodes are connected by the edges, and can be represented as a matrix;
- A graph could be:
  - Binary (Absence/presence);
  - Weighted (Connection weight);
  - Directed (A->B ≠ B->A);
  - Undirected (Bidirectional links);



Mancini and Cercignani, Fundam Inform 2018

Networks and graphs

- In the scientific literature the terms network and graph are often used interchangeably;
- However, there is a distinction between the two:
  - Network refers to the real system under study;
  - Graph refers to the mathematical representation of these systems;
- Therefore, two completely different networks could be represented by the very same graph!

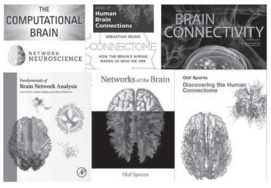


Barabasi, "Network Science" (2016)

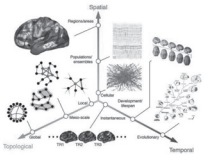
Networks and graphs

- In the very last years, network models and graph theory were used for:
  - Basketball games (Fewer et al., 2012);
  - Quantum states (Adhikari et al., 2012);
  - Medieval transactions (Villa-Vialanex et al., 2012);
  - Notarial acts (Rossi et al., 2013);
  - Heart modeling (Basavaprasad et al., 2014);
  - Labor contractions (Nader et al., 2015);
  - Pastoralist societies (Hao et al., 2015);
  - Scientific progress (!) (Barbash et al., 2015);
  - Terrorism (!!) (Manrique et al., 2016);
  - Brexit (!!!) (Petersen and Puliga, 2017);

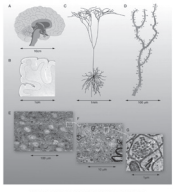
Networks and graphs … And brains!



The brain as a network



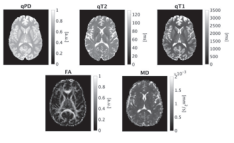
Betzel and Bassett, NeuroImage 2017



Lichtman and Denk, Science 2011

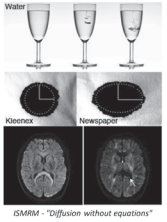
Brain structural networks at the macroscale

- Structural networks are estimated using different MRI modalities;
- We need at least two modalities because:
  - One modality needs to be able to accurately represent grey matter;
  - Another one needs to be able to infer about white matter organization;
  - As we will see, additional modalities can be integrated to model more complex properties;
- The first modality required to accurately represent cortical areas is given by T1-weighted data;



Diffusion MRI

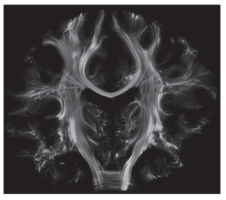
- To study white matter organization, the primary method is given by diffusion MRI, which allows to estimate diffusion phenomena of water molecules;
- Different volumes are acquired to measure the diffusion of the water in several directions;
- White matter fibers constitute an anisotropic medium, allowing to identify the main diffusion direction and therefore an estimation of the related orientation of the fiber;



ISMRM - "Diffusion without equations"

Diffusion MRI

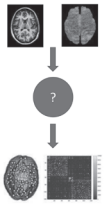
- Diffusion data can be modelled using a tensor, which is simple but unable to represent complex geometry (e.g. crossing fibers);
- More complex models, usually requiring longer protocols, can estimate a fiber orientation distribution;
- Using tractography on the modelled data, it is possible to estimate the trajectories of the white matter fibers using two classes of algorithms:
  - Deterministic: propagation of a streamline until a stop criterion is reached;
  - Probabilistic: fit of a probability distribution of the preferred direction of water diffusion;



A. Anwander, Max Planck Institut for Human Cognitive and Brain Sciences

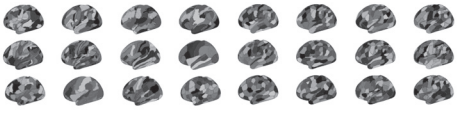
Structural networks in the brain

- In the next slides, we will go through the methodological choices to fit a network model to MRI data;
- The main steps are:
  - Definition of nodes;
  - Definition of edges;
  - Eventual definition of weights;
  - Eventual thresholding;
- Let's start by taking a look on how to define a node;
- *How hard could it be?*



Defining nodes

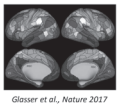
...This hard.



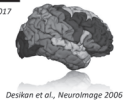
Arslan et al., NeuroImage 2018

Defining nodes: possible approaches

- Node = measurement point;
  - Pro: no assumption;
  - Con: no guarantee of any meaning behind the definition;
- Cytoarchitectonics;
  - Pro: multimodally validated;
  - Con: potential dependence on inter-individual variability;
- Macroscopic landmarks;
  - Pro: straightforward approach;
  - Con: variations in size;



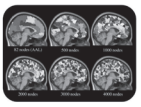
Glasser et al., Nature 2017



Desikan et al., NeuroImage 2006

Defining nodes: possible approaches

- Random parcellation;
  - Pro: spatial homogeneity;
  - Con: no guarantee of any meaning behind the definition;
- Landmarks+random:
  - Pro: combines the advantages of the two;
  - Con: dependency on the coarser scheme;
- Connectivity-based:
  - Pro: data-driven;
  - Con: DWI-inherited limitations;

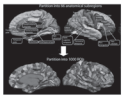


Zalesky et al., NeuroImage 2010

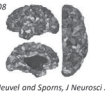
Moreno-Dominguez, Hum Brain Mapp 2014

Defining nodes: common choices

- A good approach in MRI studies is to assess the consistency of the results from a coarser (usually landmark-based) parcellation and from a finer one (usually obtained randomly parcellating the coarser one);
- Studies on the influence of parcellation on subsequent measures have shown:
  - Consistency of gross properties;
  - Potential variations in quantitative measures: cross-validation required.



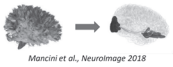
Hagmann et al., PLOS Biol 2008



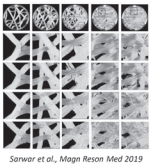
Van den Heuvel and Sporns, J Neurosci 2011

Defining edges

- The definition of edges is more straightforward, although it presents several choices and pitfalls;
- The streamlines reconstructed from tractography are used to assess if two given regions are connected or not;
- The preprocessing influences how the network will look like:
  - Tensor: unable to resolve crossing fibers, less streamlines, more conservative;
  - More complex models: more streamlines but potentially more false positives;
  - Deterministic tractography: more false negatives;
  - Probabilistic tractography: more false positives;



Mancini et al., NeuroImage 2018

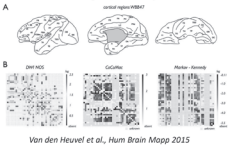


Sarwar et al., Magn Reson Med 2019

Defining edges

- As mentioned before, networks can be represented using binary or weighted graphs;
- For the brain, it means choosing if we want to model:
  - Presence/absence of an connection between two regions;
  - Different characteristics of white matter connections;
- The most common choice is the latter; however, it is important to remark that the number of streamlines is NOT a quantitative measure!
- Comparisons between DWI and tract tracing data in the macaque showed in any case good agreement in terms of graph-based measures;

Mancini and Cercignani, Fundam Inform 2018



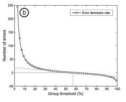
Van den Heuvel et al., Hum Brain Mapp 2015

Shen et al., NeuroImage 2019

Defining edges

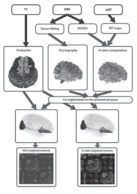
- Two additional aspects worth to discuss are alternative weights and thresholding:
  - It is possible to use other measures obtained from MRI (e.g. FA, MTR, g-ratio), map them to the streamlines and use them as weights; in this way, specific properties are used to characterize the edges (e.g. myelination);
  - Given the presence of false positives, a common approach to reduce them is to threshold the resulting connectivity matrix;

Mancini et al., NeuroImage 2018

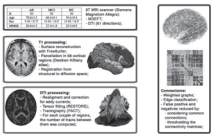


De Reus and Van den Heuvel, NeuroImage 2013

Processing pipelines



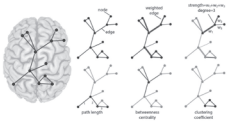
Mancini et al., NeuroImage 2018



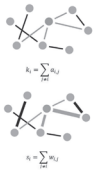
Mancini et al., IEEE ISBI 2016

Network properties and graph measures

- Once a network model has been defined, it is possible to characterize the system under study using specific measures;
- These measures can characterize a specific node in relationship to the others (local measures) or the whole network (global measures);
- We can broadly divide them in:
  - Centrality;
  - Core-periphery;
  - Paths;
  - Motifs;
  - Topology;
  - Modularity;



Graph measures: centrality



$$k_i = \sum_{j \neq i} a_{i,j}$$

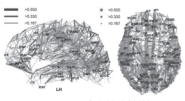
$$s_i = \sum_{j \neq i} w_{i,j}$$

Mancini and Cercignani, Fundam Inform 2018

- The simplest measure to characterize a node in a network is the degree, which is the number of its connections;
- For weighted networks, it is possible to generalize it to its strength, which takes into account the weights of its connections;
- It is also the simplest measure of centrality, quantifying the influence of a node on the rest of the network;
- An important fist observation is that most real-world networks do not present a uniform degree distribution;

Graph measures: centrality

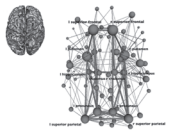
Barabasi, "Network Science" (2016)



Hagmann et al., PLOS Biol 2008

- This consideration leads us to a first important property for networks: the presence of hubs, a limited number of nodes with a high degree/strength;
- It is important to notice that there are other centrality measures and so hubs can be defined in more than one way;
- Sporns and colleagues (PLOS One 2007) have showed that hub regions in the brain show consistently high values in different centrality measures;
- A meta-analysis by Crossley and colleagues (Brain 2014) has shown how hubs are involved in most neurological disorders;

Graph measures: core-periphery



Van den Heuvel and Sporns, J Neurosci 2011

- Not only the brain has hubs, but also these hubs are highly interconnected with each other, the so-called rich-club phenomena;
- As a result, the nodes are classified in hubs and peripherals, while the connections can be classified in rich-club, feeder or local ones;
- Simulations have shown that rich-club nodes mediate the majority of the information flow;
- Robustness analysis shows how targeted attack to these nodes has a dramatical effect in terms of disruption;

Graph measures: paths and motifs

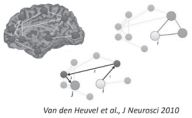
$$L = \frac{1}{n}\sum_{i \in N} L_i = \frac{1}{n(n-1)}\sum_{i \neq j} l_{ij}$$

$$C_i = \frac{2t_i}{k_i(k_i-1)}$$

Mancini and Cercignani, Fundam Inform 2018

- Two common properties to further characterize a network are the path length and the clustering coefficient:
  - The minimum path length between two nodes represent the minimum numbers of edges between those two nodes;
    - Low minimum path length -> "immediate communication between the nodes";
    - Its most common interpretation for the brain is in term of information integration;
  - The clustering coefficient represents the number of triplets (i.e. motifs with a triangular shape) around a node;
    - High clustering coefficient -> "the neighbors of a node are neighbors with each other";
    - Its most common interpretation for the brain is in terms of functional segregation;

Graph measures: topology

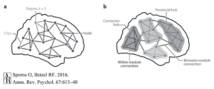


Van den Heuvel et al., J Neurosci 2010

Mancini and Cercignani, Fundam Inform 2018

- In their seminal work published on Nature (1998), Duncan and Strogatz showed that most real-world networks present a balance between in terms of short paths (typical of random networks) and high clustering (typical of regular lattices);
- This property has been called small-world and brain networks as well (both functional and structural) present this balance between integration and segregation;
- Small-worldness can be computed as the ratio between the clustering and path length measures;
- Since it offers a very global summary, it is always necessary to complement it with considerations at the nodal and connectional level;

Graph measures: modularity



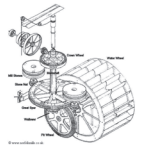
Sporns and Betzel, Annu Rev Psychol 2016

Van den Heuvel and Sporns, Trends Cogn Sci 2013

- Modules are encountered across a broad range of networks (e.g. groups of individuals in social networks, ensembles of interacting proteins, coregulated genes in cellular networks);
- There is large evidence of modules in the brain from both the structural and the functional perspectives;
- Modules derive from a decomposition of the network into subcomponents that are internally strongly coupled, but externally only weakly coupled;
- Modular organization offers an additional layer in the hub structure;

Mind the assumptions!

- Historically, several analogies have been drawn between the brain and trending ideas;
- It is not surprising then that the current trend is to apply information theories and social network concepts to the study of the nervous systems;
- As a result, there's the risk of "falling in love" with the analogy forgetting about the actual brain;



Mind the assumptions!

- " The first set of graph theory applications in brain network analysis was limited to exploring conventional network metrics borrowed from social networks, including degree distribution, clustering, path length, small world, efficiency, etc. While these studies were highly revealing, they mainly borrowed tools and concepts from social network theory and did not leverage principles learned from the specific neuroscientific context of these networks. More recent work has begun to explore bottom-up network modeling of various brain phenomena." (A. Raj, 2012)
- Several articles calculated several graph measures looking for biomarkers without actually interpreting what these measures meant;

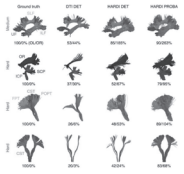
Interpretation issues

- In the case of characteristic path length, the a priori hypothesis is that shortest paths take in a large fraction of network traffic load;
- Although clustering is a measure of how densely neighbor nodes are interconnected, the implications for local processing are not straightforward;
- Global measures as the average clustering coefficient and the characteristic path length can hardly show significant differences in case of brain stimulation;
- In case of local measures as the degree and the strength, as long as the connections are measured in a clear way they make more sense and they are easier to interpret.

xkcd.com

Pitfalls inherited from diffusion MRI

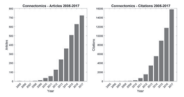
- Diffusion MRI suffers of several limitations that are inherited by the network models build on top of it:
  - Diffusion-based measures are influenced by several factors (axonal number, density, caliber, myelination);
  - Head-motion, physiological noise and imaging artifacts act as additional confounding factors;
  - Diffusion tractography has difficulty reconstructing long-range pathways;
  - Diffusion tractography cannot resolve source and target of a connection -> only undirected graphs;



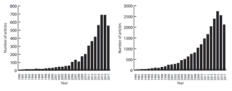
Maier-Hein et al., Nat Comm 2017

Translation into clinical applications

- The most common use of network models in neuroimaging has been first in neuroscience, in particular fundamental research;
- However, as the popularity of this approach has increased, there has been a constantly increasing number of studies in clinical populations;
- For epilepsy, the major applications in the literature so far include:
  - Getting new insights into different manifestations of epilepsy;
  - Identifying biomarkers for surgical planning;
  - Performing network-based simulations to understand seizure propagation;
  - Starting to build multimodal patient-specific models;

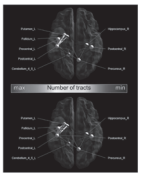


Sporns and Bassett, Netw Neurosci 2018



Mancini and Cercignani, Fundam Inform 2018

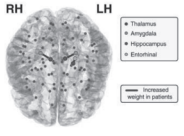
Applications: getting new insights



Caeyenberghs et al., NeuroImage Clin 2014

- Caeyenberghs and colleagues compared a group of 34 Juvenile myoclonic epilepsy (JME) patients and matched controls;
- They observed significant differences in a subnetwork involving primary motor, parietal and subcortical regions;
- They also observed significant correlation between structural connectivity and cognitive task performance;
- These findings suggest that JME-related changes extend beyond the frontal lobes;

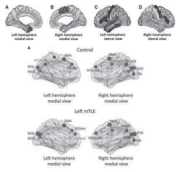
Applications: getting new insights



Taylor et al., NeuroImage 2015

- Taylor and colleagues looked at how reduced volumes and connectivity changes are related in temporal lobe epilepsy (TLE) patients;
- Although they found several differences in terms of surface area between patients and controls, they observed more subtle changes in terms of connectivity;
- It must be noticed that the authors did use connection-wise comparisons and appropriate multiple corrections;
- Nevertheless, these results suggest that it is important to look at potential relationships between atrophy and altered connectivity;

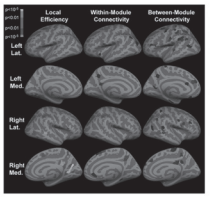
Applications: getting new insights



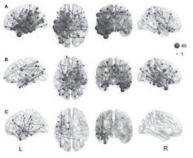
Liu et al., Epilepsia 2014

- Liu and colleagues characterized in terms of centrality the structural networks reconstructed from mesial temporal lobe epilepsy (mTLE) patients;
- They observed decreased efficiency in ipsilateral temporal, bilateral frontal and bilateral parietal areas;
- They also observed alterations in terms of hubs;
- These results seem to describe from a different perspectives the structural abnormalities previously observed in gray and white matter in mTLE;

Applications: getting new insights

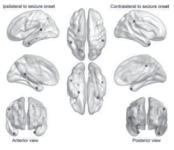


DeSalvo et al., Radiology 2014



Besson et al., NeuroImage 2014

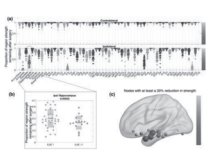
Applications: biomarkers for surgical planning



Bonilha et al., Neurology 2013

- Bonilha and colleagues retrospectively studied medial temporal lobe epilepsy (MTLE) patients who underwent temporal lobectomy;
- Using specific measures, they were able to distinguish seizure-free patients from the others;
- Non-seizure-free patients exhibited higher medial-lateral temporal and temporal-parietal connectivity;
- These results suggest the idea that structures traditionally not removed during surgery may be associated with seizure control;

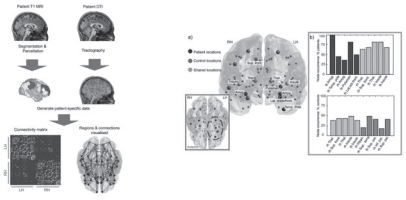
Applications: biomarkers for surgical planning



Taylor et al., NeuroImage Clin 2018

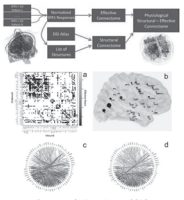
- Taylor and colleagues proposed a pipeline to estimate the structural white matter network and classify patients in seizure free or not;
- To do this, they trained their classificator using both network-based and volumetric measures;
- They observed that surgery leads to patient-specific reductions in efficiency and strength;
- This result suggest that given a resection mask it is possible to use this approach during planning;

Applications: network-based simulations



Hutchings et al., PLOS Comp Biol 2015

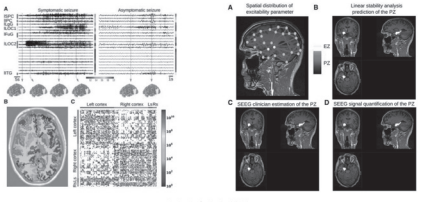
Applications: patient-specific models



Donos et al., NeuroImage 2016

- Donos and colleagues proposed a method to estimate the effective functional connectivity;
- They combined intracranial electrical stimulation with diffusion data in order to build a "physiological structural-effective connectome";
- Although still at the population level, this approach lays the ground for building patient-specific models, allowing for accurate simulation-based surgical planning;
- It provides also a framework for further study of structure-function relationship in epilepsy;

Applications: patient-specific models



Proix et al., Brain 2017

Take-home message

- Recent literature has shown how network models are an effective way to study the human brain;
- There is a great potential (partially unexploited) in using this approach for multimodal analysis and for simulations;
- Epilepsy is ultimately a disease of functionally and structurally aberrant connections, so it constitutes an ideal fit for network models;
- One should keep in mind that in network analysis the attention is focused on how network elements interact with each other, so:
  - It is different from seed-based analysis;
  - It is different from independent components;
  - The outcomes must be interpreted taking into account which measure has been modeled to connect the nodes of the graph;

xkcd.com

Thank you for your attention!

Questions?

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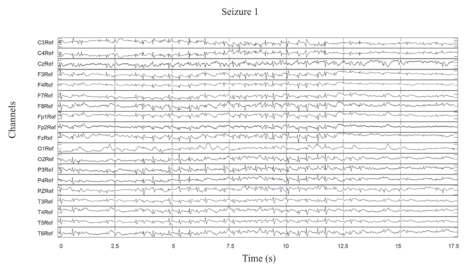
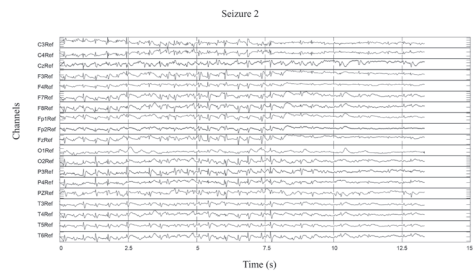
Christophe Bernard (France)

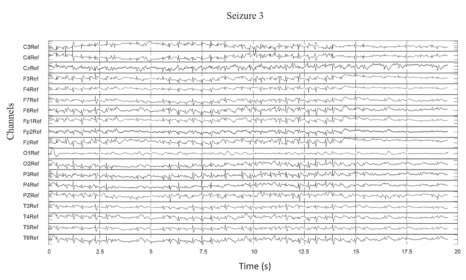
CONNECTOMES: WHAT CAN THEY TELL US ABOUT BRAIN FUNCTION AND DYSFUNCTION

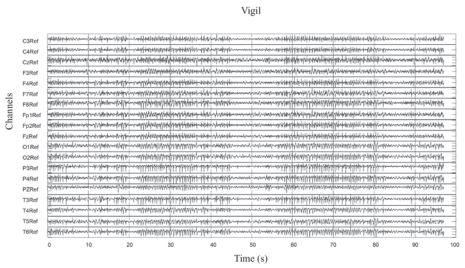
Jean Faber (Brazil)

FROM ELECTROPHYSIOLOGICAL RECORDINGS TO CONNECTIVITY: HOW TO CONSTRUCT A GRAPH AND HOW TO ANALYZE IT?








Vigil
C3Ref
C4Ref
CzRef
F3Ref
F4Ref
F7Ref
F8Ref
Fp1Ref
Fp2Ref
FzRef
O1Ref
O2Ref
P3Ref
P4Ref
PZRef
T3Ref
T4Ref
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Channels
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10
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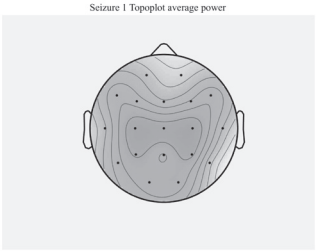
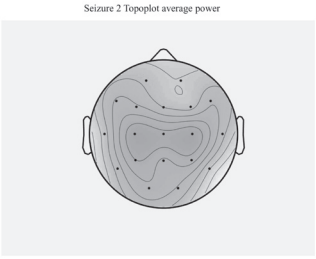
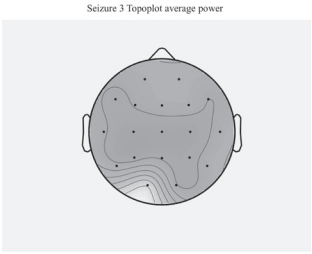
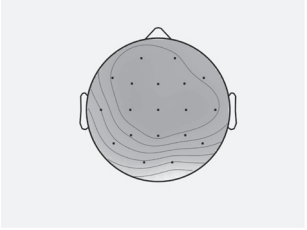
30
40
50
60
70
80
90
100
Time (s)


Seizure 1 Topoplot average power


Seizure 2 Topoplot average power

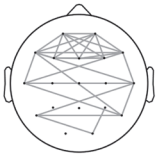

Seizure 3 Topoplot average power

Seizure 1 Connected average power

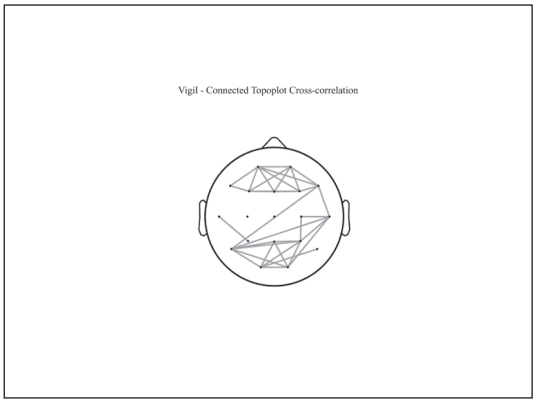


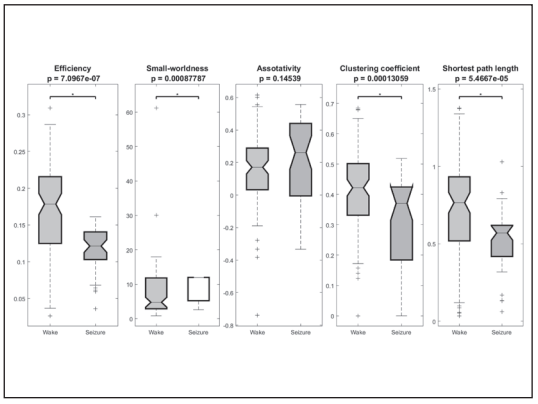
Seizure 1 - Connected Topoplot Cross-correlation

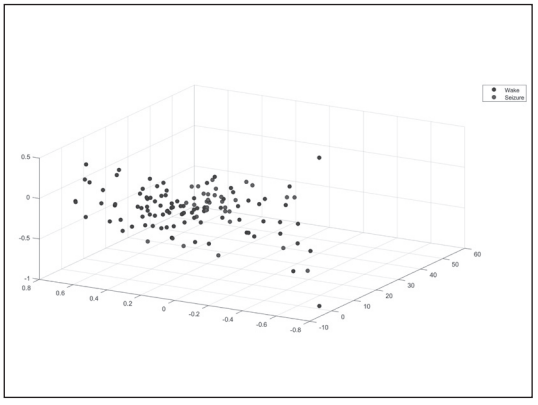
Seizure 2 - Connected Topoplot Cross-correlation

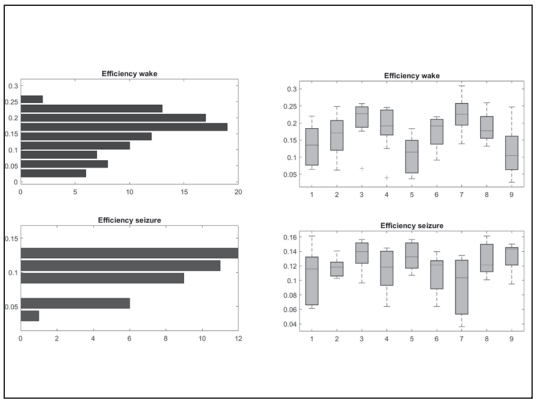


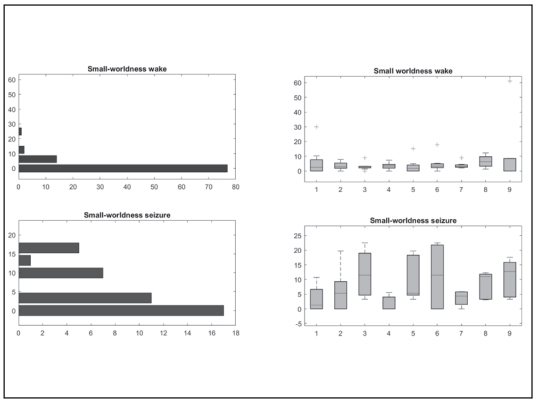
Seizure 3 - Connected Topoplot Cross-correlation

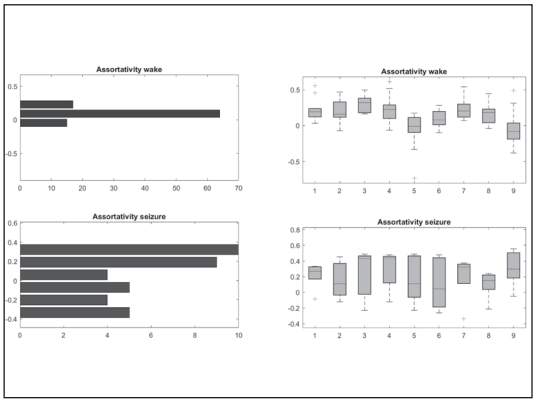

Vigil - Connected Topoplot Cross-correlation

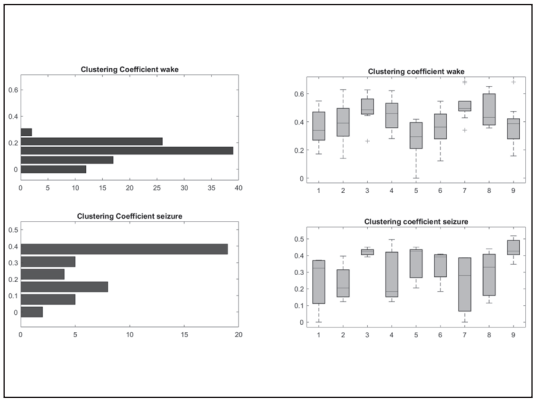

Efficiency
p = 7.0967e-07
Small-worldness
p = 0.00087787
Assotativity
p = 0.14539
Clustering coefficient
p = 0.00013059
Shortest path length
p = 5.4667e-05
Wake
Seizure

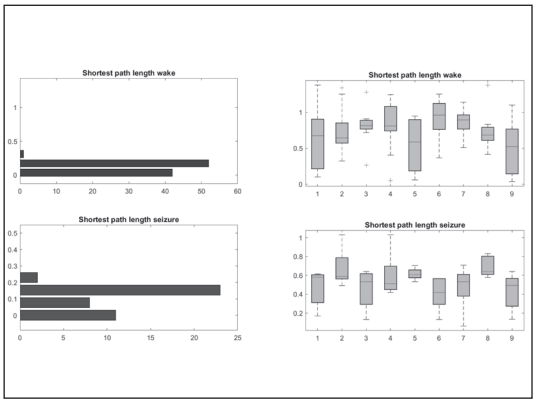

Wake
Seizure


Efficiency wake
Efficiency wake
Efficiency seizure
Efficiency seizure


Small-worldness wake
Small worldness wake
Small-worldness seizure
Small-worldness seizure


Assortativity wake
Assortativity wake
Assortativity seizure
Assortativity seizure


Clustering Coefficient wake
Clustering coefficient wake
Clustering Coefficient seizure
Clustering coefficient seizure


Shortest path length wake
Shortest path length wake
Shortest path length seizure
Shortest path length seizure

Edson Amaro (Brazil)

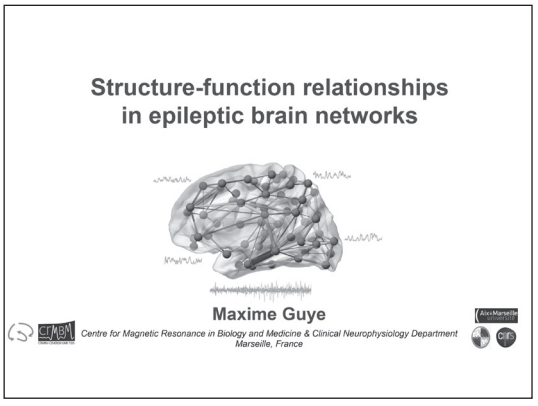
NEUROIMAGING AND BIG DATA: BEYOND THE 'OMICS' REVOLUTION

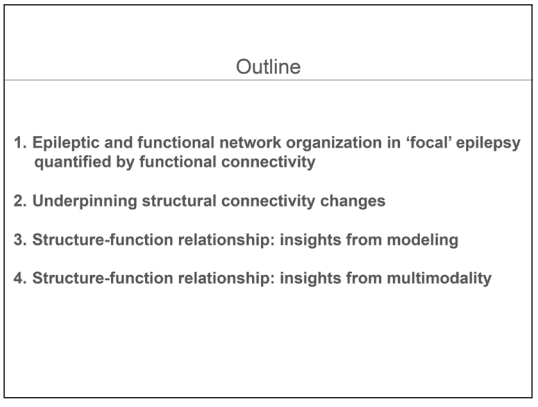
Adriano Tort (Brazil)

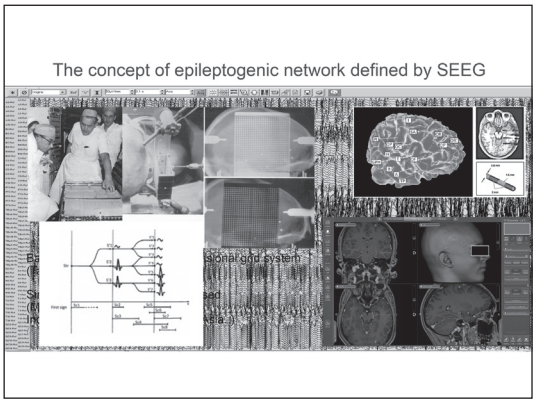
NOVEL INSIGHTS INTO THE MECHANISMS OF SPATIAL CODING BY HIPPOCAMPAL NEURONS

Maxime Guye (France)

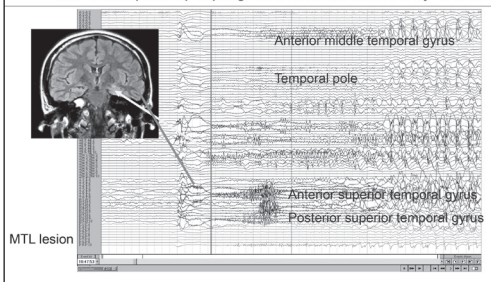
STRUCTURE-FUNCTION RELATIONSHIPS IN EPILEPTIC NETWORKS



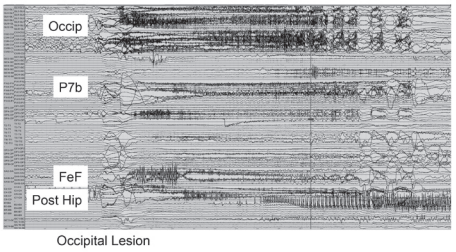




The concept of epileptogenic network defined by SEEG



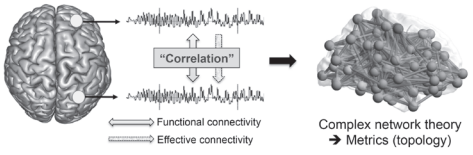
The concept of epileptogenic network defined by SEEG



Occipital Lesion

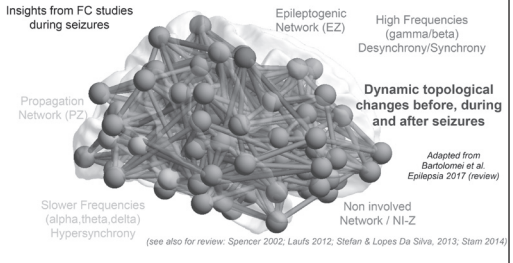
Quantifying Functional Connectivity

➔ Statistical interdependencies between remote neurophysiological events
Neuronal spiking *(Aertsen et al. J Neurophysiol 89)*
Time series (EEG, iEEG (ECoG,SEEG), MEG,fMRI) *(Friston Prog Neurobiol 02)*



➔ Quantitative study: from pair-wise connectivity to network communication analyses
(Varela et al. Nat Rev Neurosci 01, Uhlhaas & Singer Neuron 06, Guye et al. Curr Op Neurol 08, Sporns Nat Rev Neurosci 14…)

Network organization of focal onset seizures

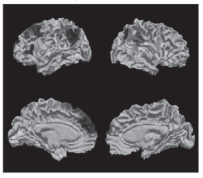


(see also for review: Spencer 2002; Laufs 2012; Stefan & Lopes Da Silva, 2013; Starn 2014)

Quantifying Functional Connectivity by using fMRI

Connectivity of large-scale brain networks

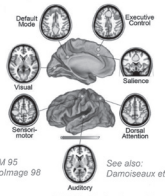
Resting-state fMRI



Statistical dependency of spontaneous slow fluctuation of BOLD ➔ FC

See also:
Biswal et al. MRM 95
Lowe et al. NeuroImage 98

➔ Quantification of RSNs

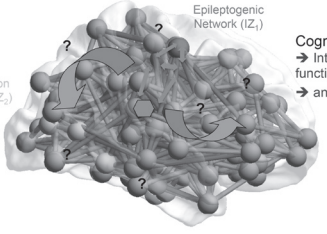


See also:
Damoiseaux et al. PNAS 06

Raichle Brain Conn 11

What can we learn from interictal connectivity?

EN Localisation ➔ specific interrelations within epileptic networks?



Cognitive impairment
➔ Interrelations within functional networks?
➔ and impact of EZ?

Interictal FC within the Epileptogenic Network

Hypo or hyper functional connectivity?

Pair-wise functional connectivity analysis

ECoG/SEEG (/MEG/EEG): Increased FC *(Schevon et al. 2007, Bettus et al. 2008, Ortega et al. 2008, Palmigiano et al. 2012, Bartolomei et al. 2013; Antony et al. 2013, Engiot et al. 2015, Vecchio et al. 2015, Iamotti et al. 2016, Lagarde et al. 2018...)*

RsfMRI: Decreased FC *(Bettus et al. 2009, 2010, 2011; Weaver et al. 2013)*
Increased FC (local) *(Morgan et al. 2010, Stufflebeam et al. 2011)*

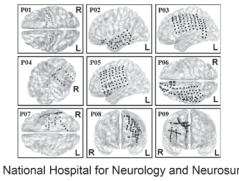
Network communication analysis

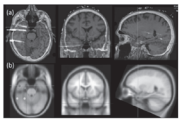
Increased local and decreased global network efficiency *(Bartolomei et al. 2013,17; van Diessen et al. 2014...)*
Important role of hubs (Centrality, Degree) *(Wilke et al. 2011; Ridley et al. 2015; Courtens et al. 2016; Nissen et al. 2017...)*

Interictal FC within the Epileptogenic Network

Hypo or hyper functional connectivity?

Insights from simultaneous intracranial EEG-fMRI





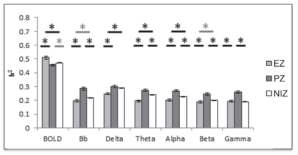
Ridley et al. Brain Topography 2017

National Hospital for Neurology and Neurosurgery
UCL University College London Hospitals NHS
NHS Foundation Trust

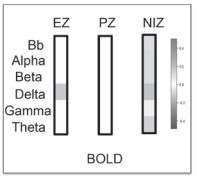
Interictal FC within the Epileptogenic Network

Insights from simultaneous intracranial EEG-fMRI

FC in different modalities and zones



Intermodal correlation



Ridley et al. Brain Topography 2017

Inter-modality discrepant FC within epileptic but not within normal areas

Interictal FC in Functional Networks

Hypo or hyper functional connectivity / Links with cognitive impairments?

Pair-wise functional connectivity analysis in TLE

• BOLD *(Constable 2013; Centeno & Carmichael 2014; Pittau & Vulliemoz 2015, Englot et al. 2016 for reviews)*

➔ Decreased FC within functional networks and links with cognitive dysfunctions: Language (pre/post-surgical), Memory, Attention, Perception, DMN, Depression, Motor…

(Waites et al. Ann Neurol 06, Bonelli et al. Epilepsia 12; Addis et al. Brain 07, Campo et al. CerCor 12, Voets et al. J Neurosci 14, Douw et al. PloS one 15, Doucet et al. HBM 16; Zhang et al. Neurosci Lett 09, Zheng et al. Epi Res 12; Zhang et al. J Neurol 09; Zhang et al. Brain Res 10, Liao et al. HBM 11, Widjaja et al. AJNR 13, Rektor et al. Brain Topogr 13; Kemmotsu et al. Epi Behav 13, Doucet et al. Brain Cog 13 ;Rektor et al. Brain Topogr 13, Haneef et al. Epilepsia 14…)

Links with duration and severity of epilepsy *(Liao et al. HBM 11; Voets et al. Brain 12; Morgan Epi Res 15)*

➔ Increased FC within functional networks and links with (mal)adaptive processes:

- Contralateral / working memory *(Bettus et al. JNNP 10, Campo et al. Neuroimage 13, Holmes HBM 12)*
- Propagation areas? *(Haneef et al. Epilepsia 14; Barron NI Clin14; Wirsich et al. NI Clin16)*

• EEG/MEG/iEEG *(Englot et al. Brain 15, Epilepsia 16; Colto et al. Epilepsia 16, Lagarde et al. Brain 18)* ➔ Similar patterns

Interictal FC in Functional Networks

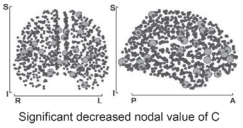
Hypo or hyper functional connectivity / Links with cognitive impairments?

Topological pattern analysis of FC in TLE

Loss of network efficiency associated with cognitive decline in epilepsy

↓ Local Efficiency
↓ Global Efficiency

41 TLE
23 controls



Vlooskwijk et al. Neurology 11

Significant decreased nodal value of C

Interictal FC in Functional Networks

Hypo or hyper functional connectivity / Links with cognitive impairments?

Topological pattern analysis of FC

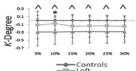
• BOLD 33 patients (19 TLE) → 19 right et 14 left
17 controls

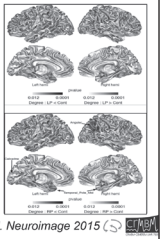
Patients vs controls: No significant ≠

Whole brain alteration in left epilepsy

↓ Eloc, Eglob, modularity

Hub reorganization in right epilepsy



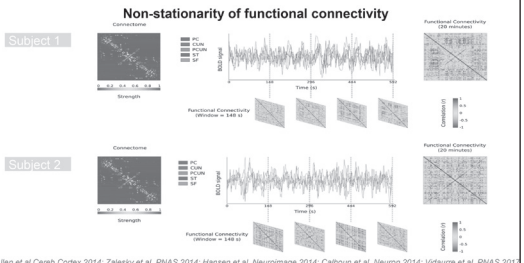


➔ Influence of epilepsy lateralisation:
≠ between R and L-TLEs for FC hippocampus vs whole brain

(Peixeira et al. BMC Neuro 10; Morgan et al. Epilepsia 12; Pittau et al. Epilepsia12) *Ridley et al. Neuroimage 2015*

Are usual functional connectivity metrics adapted?

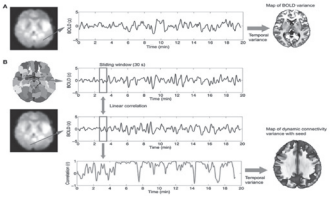
Non-stationarity of functional connectivity



Allen et al Cereb Cortex 2014; Zalesky et al. PNAS 2014; Hansen et al. Neuroimage 2014; Calhoun et al. Neuron 2014; Vidaurre et al. PNAS 2017

Dynamic Functional Connectivity

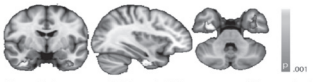
What does the variance of FC can tell us about epileptic processes?



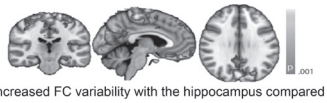
Laufs et al. Front Neurol 2014

Dynamic Functional Connectivity

Voxel-based variance maps in rsfMRI



Significantly increased FC variablitiy compared to controls



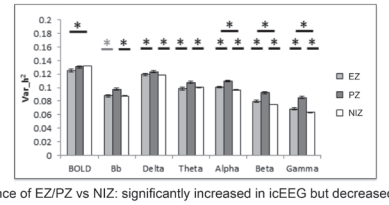
Significantly increased FC variability with the hippocampus compared to controls

Laufs et al. Front Neurol 2014

Dynamic Functional Connectivity

Variance maps in rsfMRI

Insights from simultaneous intracranial EEG-fMRI



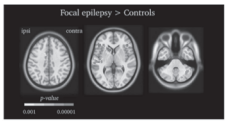
FC variance of EZ/PZ vs NIZ: significantly increased in icEEG but decreased in BOLD

Ridley et al. Brain Topography 2017

Dynamic Functional Connectivity

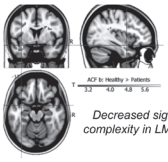
Beyond variance – Other approaches

Increased or decreased signal variability?



Increased signal variability of local connectivity in neocortical epilepsy

Pedersen et al. Neuroimage 2017



Decreased signal complexity in LMTLE

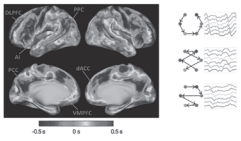
Nedic et al. BMC neurol 2015

Graph less dependent of dynamics *Chiang et al. Neuroimage 2016*

Dynamic Functional Connectivity

Time lag structure in rsfMRI

Highly reproducible lag structure and temporal sequences (threads)

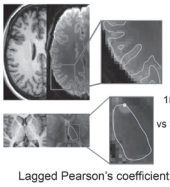


Modified with appropriate focality by task paradigms

Mitra et al. J Neurophysiol 2014, PNAS 2015

See also: Vidaurre et al. PNAS 2017

High-res rsfMRI



15 patients (SEEG) vs 36 controls

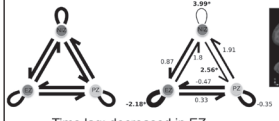
Lagged Pearson's coefficient

Bandt et al. (submitted)

Dynamic Functional Connectivity

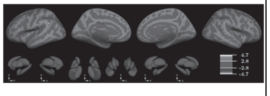
Time lag structure in rsfMRI

Region-based approach



Time lag: decreased in EZ but increased in non epileptic regions

EZ-seeded approach



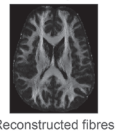
Both increased and decreased time lag between EZ and other regions
➔ RSNs

Bandt et al. (submitted)

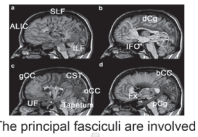
Structural Connectivity Alterations

Structural connectivity is disrupted well beyond the epileptogenic regions

Diffusion-weighted MRI



Reconstructed fibres
➔ SC



The principal fasciculi are involved

Link with cognitive impairments
DTI correlates (MD,FA…) of language, memory…

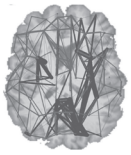
(Ahmadi, et al. 2009; Campos, et al. 2015; Concha, et al. 2005; Concha, et al. 2007; Diehl, et al. 2008; Ellmore, et al. 2009; Govindan, et al. 2008; Hagler, et al. 2009; Kim, et al. 2008; Liao, et al. 2010; Lin, et al. 2008; McDonald, et al. 2008a; Nilsson, et al. 2008; Powell, et al. 2007; Riley, et al. 2010; Rodrigo, et al. 2007; Rodrigo, et al. 2008, Bonilha, et al. 2010; Voets, et al. 2015…)

Do structural alterations explain functional connectivity?

Graph analyses of structural connectivity

Alterations linked to cognitive impairments

↓ Global and local efficiency in patients with cognitive impairments only



DTI
39 Patients
23 controls

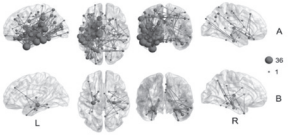
Vaessen et al. Cereb Cortex 2011
See also: Liu et al. Epilepsia 2014

Fiber density / cognitive scores correlations

Do structural alterations explain functional connectivity?

Whole brain approach of pair-wise structural connectivity

Patterns of disconnection in Left and Right MTLE

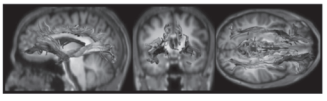


Besson et al. Neuroimage 2014
See also: Fang et al. Neuroimage Clin 2015

Does structural connectivity shape seizure organisation?

Link with propagation?

Differences among epilepsy types



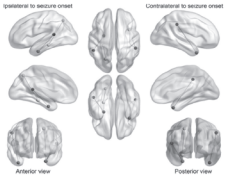
≠ patterns of 4 propagation bundles (FA, AD, RD)
In 17 TLE (-)MRI, 17 TLE (HS),14 FLE (FCD)

Campos et al. Epilepsia 2015

Does structural connectivity shape seizure organisation?

Link with propagation? Predictive factor?

Patterns depending on surgical outcomes



20 Patients
18 Controls

Bonilha et al. Neurology 2013

Higher fiber density in non-seizure free

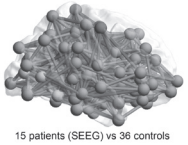
See also: Dinkelacker et al. Epilepsia 2015

Does structural connectivity shape seizure organization?

Whole-brain high-resolution structural connectivity

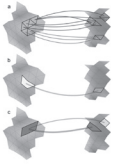
Comparison with SEEG labels

Zone-based analysis (EZ, PZ, NIZ)



15 patients (SEEG) vs 36 controls

Whole-brain analysis



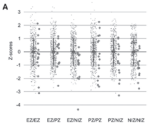
Besson et al. Brain 2017 *(see also Besson et al. NeuroImage 2014, Brain Topography 2017)*

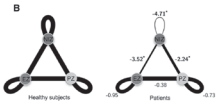
Does structural connectivity shape seizure organization?

Whole-brain high-resolution SC – group analysis

Anatomic consistencies

Zone-based approach (EZ, PZ, NIZ)





➔ SC is maintained in epileptic networks but decreased in other regions

Besson et al. Brain 2017

Does structural connectivity shape seizure organization?

Whole-brain high-resolution SC – group analysis

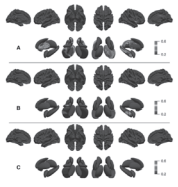
Anatomic consistencies

Whole-brain approach

Overlap with RSNs

Overlap with EZ

Overlap with PZ



➔ Specific large-scale RSNs are more consistently affected

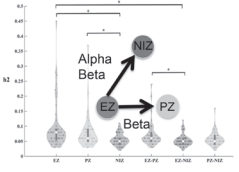
Functional network	Network involvement index (%)	Proportion of network involved (%)
Salience	31.8	13.5
Default mode	25.2	6.9
Frontoparietal control	19.0	9.1
Somatomotor	11.7	3.9
Basal frontal/temporopolar	12.4	7.9
Visual	0.4	0.1
Dorsal attention	0	0

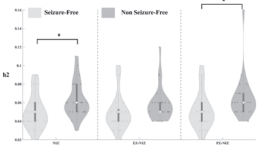
Besson et al. Brain 2017

Concordant pattern of intrinsic functional connectivity

Interictal SEEG

Functional and effective connectivity in 59 patients with FCD





➔ EZ & PZ have higher intrinsic FC than within NIZ
➔ EZ is more connected with PZ than with NIZ

Lagarde et al. Brain 2019

Structure-function relationship – Computational models

The virtual epileptic patient

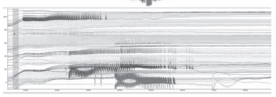
Link between structural connectivity and seizure organization

Factors relevant for seizure propagation evaluated in The Virtual Brain

Individual connectome

Virtual epileptic patient "Epileptors" placed in TVB

Jirsa et al. Brain 2014
Proix et al. JNS 2014
Jirsa et al. NeuroImage 2016

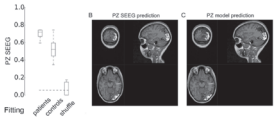


Virtual seizure dynamic fitting real seizures

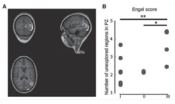
Does structural connectivity shape seizure organization?

The virtual epileptic patient

Link between structural connectivity and seizure propagation



➔ Connectome-based virtualization predict propagation zone for 15 patients



➔ Concordance with model prediction is associated to better surgical outcome

Proix et al. Brain 2017

(see also: Bonilha et al. 2015; Baker et al. 2015; Munsel et al. 2015; Gleichgerrcht et al. 2018)

Clinical Benefit of VEP: Multicentric Clinical Trial in France

Improving EPilepsy surgery management and progNOsis using Virtual brain technology (EPINOV)

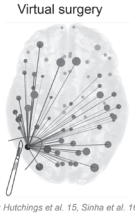
- **Improve SEEG interpretation and better guide surgical strategies in order to improve surgical outcome**
- Advance **individualized *in silico* brain modeling** (The Virtual Brain, Virtual Epileptic Patient)
- Validate our approach in a **prospective large multicenter clinical study** (N=356)
- Develop **a virtual brain-based simulation software prototype** (industrial partnership Dassault Systèmes)

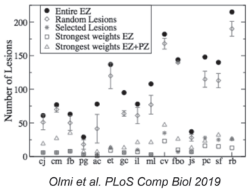
Potential impact on surgical strategies

The virtual epileptic patient

Minimizing invasiveness

Virtual surgery





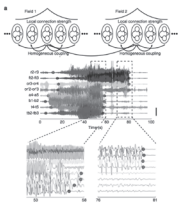
Olmi et al. PLoS Comp Biol 2019

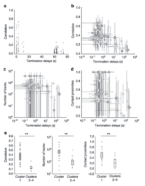
(See also: Hutchings et al. 15, Sinha et al. 16, Goodfellow et al. 16, Khambhati et al. 16, Lopes et al. 17, Steimer et al. HBM 17)

Does structural connectivity shape seizure organization?

The virtual epileptic patient

Link between structural connectivity and seizure organization

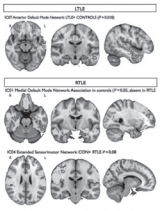




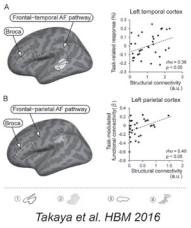
Proix et al. Nat Com 2018

Structure-function relationship of brain connectivity

Evidences from multimodality



Voets et al. Brain 2012



Takaya et al. HBM 2016

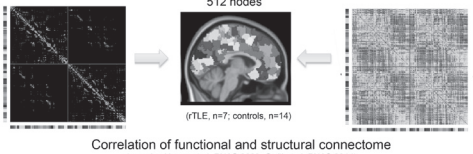
Structure-function relationship of brain connectivity

Whole brain network communication analyses

DTI
➔ SC

Common space
512 nodes

rsfMRI
➔FC



(rTLE, n=7; controls, n=14)

Correlation of functional and structural connectome

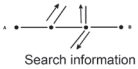
(see also Honey et al. PNAS 2009, Goñi et al. PNAS 2014)

Wirsich et al. Neuroimage Clin 2016

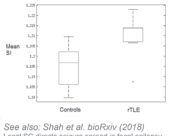
Structure-function relationship of brain connectivity

Network communication analyses

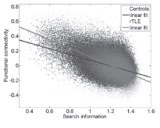
Search Information:
(Goñi et al., PNAS 2014)



Search information increased in rTLE
(higher randomization of connections)



Search information is more correlated
to functional connectivity in rTLE



See also: Shah et al. bioRxiv (2018)
Local SC directs seizure spread in focal epilepsy

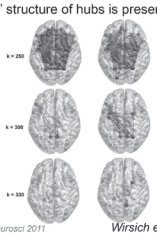
Wirsich et al. Neuroimage Clin 2016

Structure-function relationship of brain connectivity

Network communication analyses

"Rich-club" structure of hubs is preserved in rTLE

Controls



Patients

Links with propagation?

See also: Dinkelacker et al. Epilepsia 2015
Keller et al. Ann Neurol 2015

See also: van den Heuvel et al. J Neurosci 2011

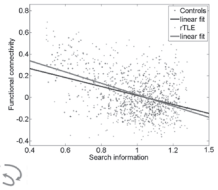
Wirsich et al. Neuroimage Clin 2016

Structure-function relationship of brain connectivity

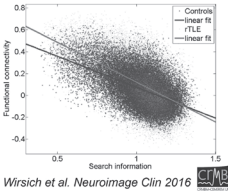
Network communication analyses

Same structure-function correlation in rich club but not in periphery

Rich club connections



Peripheral connections



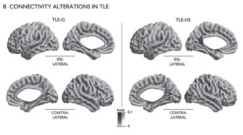
k=250

Wirsich et al. Neuroimage Clin 2016

Structure-function relationship of brain connectivity

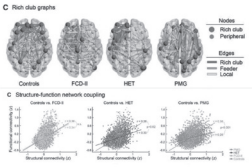
Role of the epileptogenic lesion

TLE (with vs without HS)



Bernardht et al. Ann Neurol 2016
See also Bernardht et al. Brain 2016

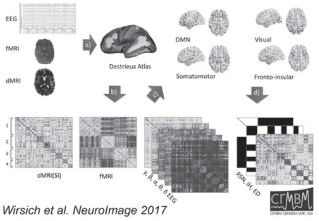
Malformations of cortical development



Hong et al. Brain 2017,
See also Hong et al. Brain 2019 (FCD subtypes)

Insights from trimodal data fusion

Complementary contributions of concurrent EEG and fMRI connectivity for predicting structural connectivity

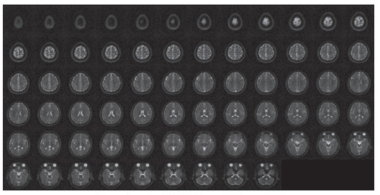


See also: Wirsich et al. bioRxiv (2018)
Concurrent EEG- and fMRI-derived functional connectomes exhibit linked dynamics

Wirsich et al. NeuroImage 2017

Ionic Homeostasis Alterations

Combining Sodium MRI and SEEG

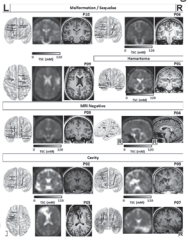


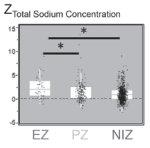
10 patients (SEEG) vs 27 controls

Ridley et al. NeuroImage 2017

Ionic Homeostasis Alterations

Combining Sodium MRI and SEEG





N=9, excluding patient who had seizure (P04)

Intercital increased TSC *(see also Wang et al. Epilepsia 1996)*

Ictal decreased TSC (P04)

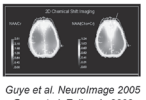
Ridley et al. NeuroImage 2017

Ionic Homeostasis, Metabolism and Neurotransmission

Neuronal-glial impairment

Insights from multimodal neuroimaging

Mitochondrial dysfunction (MRSI)

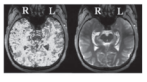


Guye et al. NeuroImage 2005
Guye et al. Epilepsia 2002

Glucose uptake (FDG-PET)

Duncan et al. Lancet Neurol 2016

Glutamate concentration (CEST)



Davis et al. Sci Transl Med 2015

Sodium homeostasis and neuron-glia interaction / metabolism

Chatton et al. Glia 2016

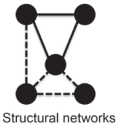
GABA concentration (MRS)

Levy & Degnan AJNR 2013

Conclusion – Structural Connectivity Organization

Insights from whole-brain high-resolution tractography and SEEG

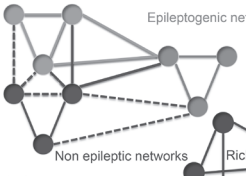
Cognitive impairments



Structural networks (RSNs)

Propagation networks

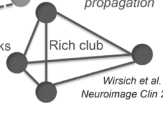
Epileptogenic network



Seizures/disease propagation

Non epileptic networks

Rich club

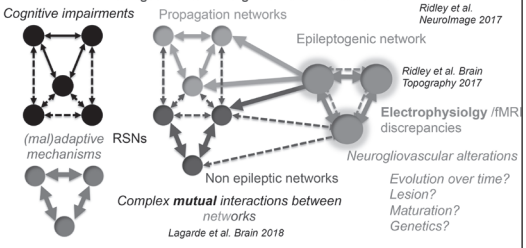


Proix et al. Brain 2017
Besson et al. Brain 2017

Wirsich et al. Neuroimage Clin 2016

Conclusion – Functional Connectivity Organization

Insights from resting-state fMRI and SEEG



Summary

Interictal Epileptic Brain Network Connectivity

- **FC:** non-specific alterations – some clinically relevant for localisation
  - ➔ Locally: rather increased FC but: signal variability, modality discrepancies
  - ➔ Globally: rather decreased FC (dysfunction) +/- (mal)adaptive increases
- **Probably shaped by structure ➔ correlated connectivity features**
- **SC:** globally disrupted but maintained in epileptic networks and core architecture of the brain (rich club) ➔ possible link with propagation and cognition
- **Common patterns but high inter-individual variability** (etiology, topography…)
- **Complex interactions between networks**
  - ➔ Isolation of EZ? Effect on (and/or of) cognition?...
- **Future directions:** models, dynamics, multimodality

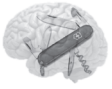
Take Home Message

Brain scaffolding plays a key role in seizure organization and associated comorbidities

Personalized computational models based on individual SC might improve surgery

epinov

Interest of multimodality to better characterise the epileptic brain



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HOW UNIQUE ARE STRUCTURAL AND FUNCTIONAL CONNECTOMES? TOWARDS PERSONALIZED MEDICINE

Mario Fales (Italy)

THE HISTORICAL BACKGROUND: BODY-BODY, BODY-MIND, MIND-BRAIN CONNECTIVITY FROM ANCIENT MESOPOTAMIA TO THE RISE OF MODERNITY

Imad Najm (USA)

MAPPING BRAIN NETWORKS IN PATIENTS WITH FOCAL EPILEPSY

Fernando Cendes (Brazil)

BRAIN CONNECTIVITY IN FOCAL ONSET EPILEPSIES

Jean Gotman (Canada)

CONNECTIVITY IN NODULAR HETEROPIA

GROUP A – CASE STUDY

/ /

Katia Lin (Brazil)

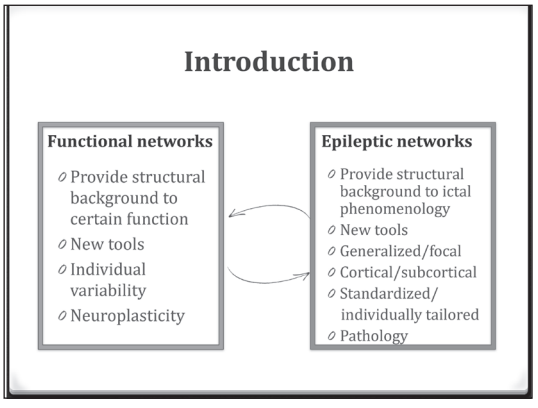
GROUP B – CASE STUDY

GROUP C – CASE STUDY

GROUP D – CONCEPTS IN NEURAL DESIGN

Patricia Braga (Uruguay)

FUNCTIONAL AND EPILEPTIC NETWORKS: BUILDING HYPOTHESIS BASED ON CLINICAL DESCRIPTIONS



Building clinical hypothesis in epilepsy patients

Functional networks

- Provide structural background to certain function
- New tools
- Individual variability
- Neuroplasticity

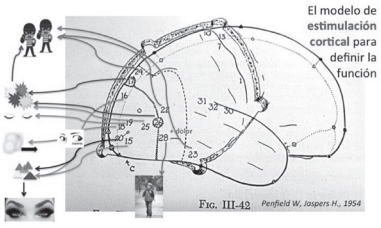
- Ictal semiology ⟶ (knowledge on functional networks) ⟶ epileptic network.
- Comprehensive evaluation ⟶ definition of epileptic network in pt. ⟶ (knowledge on functional networks) ⟶ functional risks/comorbidities.
- "Uncommon" spread patterns (ictal semiology) ⟶ "new" anatomofunctional pathways.

Epileptic networks

- Provide structural background to ictal phenomenology
- New tools
- Generalized/focal
- Cortical/subcortical
- Standardized/ individually tailored
- Pathology

Building research hypothesis

- Epilepsy as a window to brain function



Building research hypothesis in epilepsy patients based on clinical descriptions

I. Source of data

- **Interview**
  - Patient
  - Witness – relative
- Video-EEG

II. Type of information: quality

III. Type of information (temporal pattern)

- Ictal
- Post-ictal
- Pre-ictal
- Interictal /non-ictal
  - Intermittent
  - Permanent
  - Progressive
- Modifications after epilepsy treatment
  - Medications
  - Surgery
  - VNS
  - DBS

Interictal: progressive

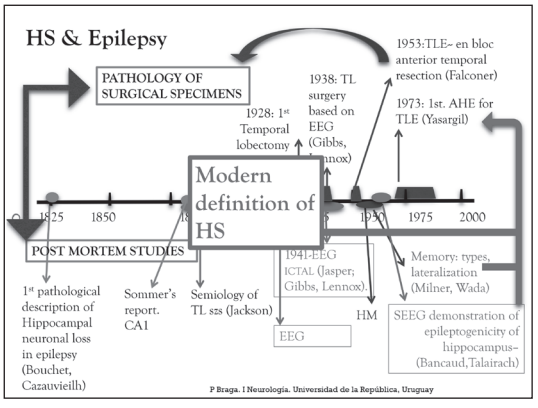
MEMORY IMPAIRMENT

Case 1

- Female, 31 years old. Rigth-handed.
- No family history of seizures.
- PMH: no history of FS. Hypothyroidism. Depression.
- Spontaneous seizures starting by 18 y.o.
- Epigastric aura followed by impairment of awareness; oral automatisms and unilateral (R) hand automatisms; sometimes left hand dystonia/left face tonic. Rare bilateral TC szs.
- Recurrent, frequent seizures
- Unresponsive to pharmacotherapy with AEDs.
- Complains of progressive memory loss along the last 2 years.

DISCOVERING MEMORIES

- **Memory functional network:**
  - Until mid XX century- it was thought to be diffuse and widespread, without clear nodes or specific brain areas.
- Memory in injured patients:
  - Frequently affected, different clinical profiles, mostly with attention deficits.
- **Memory and epilepsy:**
  - Increasing recognition of patients with suspected TLE, complaining from memory disturbances without other symptoms of cognitive decline.
  - 1953 - Henry Molaison: severe short-term and anterograde memory impairment after bilateral mesial temporal resection, as empirical treatment for his epilepsy.



NEUROPSYCHIATRY CLASSICS

Memory and the Medial Temporal Lobe: Patient H. M.

Thomas C. Neylan, M.D., Section Editor

Loss of Recent Memory After Bilateral Hippocampal Lesions

William Beecher Scoville
Brenda Milner
The Journal of Neurology, Neurosurgery and Psychiatry
(1957; 20:11–21)

Neuropsychologia, Vol. 27, No. 1, pp. 71–81, 1989.
Printed in Great Britain.

RIGHT HIPPOCAMPAL IMPAIRMENT IN THE RECALL OF SPATIAL LOCATION: ENCODING DEFICIT OR RAPID FORGETTING?

MARY LOU SMITH* and BRENDA MILNER

Department of Psychology and the Montreal Neurological Institute, McGill University, 3801 University Street, Montreal, PQ H3A 2B4, Canada

Abstract—The recall of spatial location in patients with left or right temporal-lobe lesions was studied in two experiments, in which recall was tested either immediately after presentation of an array of objects, or after an intervening verbal task, a spatial task or an unfilled interval. Deficits were found only in patients with right temporal-lobe lesions that included extensive removal of the hippocampal region, and only when recall was tested after a delay. The presence of an intervening task in the delay interval did not accentuate the deficit. The results show that, despite a normal ability to encode location, patients with large right hippocampal lesions demonstrate an abnormally rapid forgetting of such information.

Neuropsychologia, Vol. 28, No. 4, pp. 349–359, 1990.
Printed in Great Britain.

THE ROLE OF THE LEFT HIPPOCAMPAL REGION IN THE ACQUISITION AND RETENTION OF STORY CONTENT

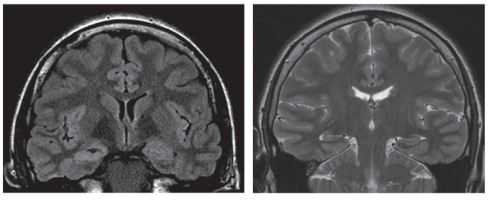
VIRGINIA FRISK* and BRENDA MILNER

Department of Psychology and Department of Neurology and Neurosurgery, McGill University and the Montreal Neurological Institute, 3801 University, Montreal, Quebec, Canada, H3A 2B4

(*Received* 27 *July* 1989; *accepted* 15 *October* 1989)

Abstract—Thirteen normal control subjects and 62 patients who had undergone either a unilateral temporal or a unilateral frontal lobectomy learned the content of a short prose passage to a strict criterion. Compared to other subject groups, patients with left temporal-lobe excisions took longer to learn the story content and, within this group, the slowest rate noted was for patients with extensive removal from the hippocampal region. When retention of the material was tested after a 20 min delay, only the group with large excisions from the left hippocampus and/or parahippocampal gyrus was impaired. This finding of abnormally rapid forgetting of material learned to criterion highlights the role of the left hippocampal region in the long-term maintenance of verbal information presented in a context.

Case 1



- Selective amygdalo-hippocampectomy in Dec 2018.
- Seizure free

MEMORY IMPAIRMENT in epilepsy

- Background
- Building hypothesis
- Current knowledge
  - New hypothesis?

Memory and epilepsy up to date

- Diagnosis of memory disturbance in epilepsy patients
- Lateralizing value of memory disturbance in MTLE
- Conclusions driven from available evaluation tools
- Prognosis
- Memory and surgical treatment

- Memory and pharmacological treatment
- Memory subtypes (paradigms) and epileptic networks
- New tools
- Other prognostic factors: complex systems

AREAS FOR RESEARCH

Pre-ictal phase

PRODROMES and PRODROMIC STATE

Prodromes: concept

- In **medicine**, a **prodrome** is an early sign or symptom (or set of signs and symptoms), which often indicate the onset of a disease before more diagnostically specific signs and symptoms develop.
- In **epilepsy**, it refers to premonitory symptoms preceding seizures in minutes, hours or even days.
- It is derived from the Greek word prodromos, meaning "running before".

Epilepsy & Behavior xxx (2018) xxx–xxx

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Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Review

Prodrome in epilepsy

Frank M.C. Besag [a,b,c,*], Michael J. Vasey [1]

Table 2
Preictal windows.

Paper	Preictal window			
	Theoretical		For study (where applicable)	
	Start	End	Start	End
Alving and Beniczky [9]	–	–	–	30 mins
Blanchet and Frommer [18]	–	–	≥24 h	Not stated
Dubois et al. [19]	3 days	30 min	–	–
Hughes et al. [12]	3 days	10 min	–	30 min
Lee and No [14]	–	–	–	1 h
Maiwald et al. [20]	–	–	–	30 min
Petitmengin et al. [8]	24 h	5 min	24 h	–
Pinikahana and Dono [15]	≥24 h	10 min	–	–
Rajna et al. [4]	30 h	1 h	–	–
Scaramelli et al. [7]	≥24 h	–	24 h	15 min
Schulze-Bonhage et al. [6]	–	–	–	30 min
Sue et al. [17]	1 week	1 h	1 week	–

Table 1
Paper summaries.

Study	% with prodrome
Alving and Beniczky [9]	2.0
Cull et al. [10]	70.9
Giuccioli et al. [11]	8.6
Hughes et al. [12]	29.1
Janz [13]	10.0
Lee and No [14]	12.1
Petitmengin et al. [8]	66.7
Pinikahana and Dono [15]	87.1
Pinikahana and Dono [16]	74.4
Rajna et al. [4]	46.6
Scaramelli et al. [7]	39.0
Schulze-Bonhage et al. [6]	6.2
Sue et al. [17]	7.2
Total	21.9

Review

Prodrome in epilepsy

Frank M.C. Besag [a,b,c,*], Michael J. Vasey [1]

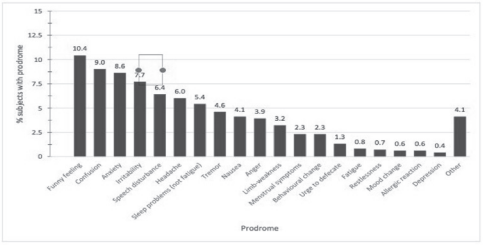

Fig. 2. Prodrome frequencies.

Seizure 18 (2009) 246–250

Contents lists available at ScienceDirect

Seizure

journal homepage: www.elsevier.com/locate/yseiz

Prodromal symptoms in epileptic patients: Clinical characterization of the pre-ictal phase

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Keywords:
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ABSTRACT

Although recent advances in seizure anticipation have been achieved with the development of several biomathematical electroencephalographic (EEG) methods, pre-ictal clinical phenomena have not been extensively investigated. The aim of the study was to thoroughly analyze premonitory or prodromal symptoms (PS) in a randomly selected sample of 100 adult epileptic patients. A semi-structured protocol was used for in-person interviews to both patients and observers. PS were found in 39% of patients, the most frequent ones being behavioral, cognitive and mood changes. Both patients with focal and generalized epilepsies reported prodromes, although they were more frequently found in the former group. PS were mostly perceived preceding complex partial and generalized tonic–clonic seizures. Prodromal symptoms were reported to have an insidious onset and their duration ranged from 30 min to several hours. The potential value of prodromes in seizure anticipation would allow the use of preventive and therapeutic measures, including drugs, neurostimulation procedures and behavioral intervention.

Seizure 18 (2009) 246–250

Contents lists available at ScienceDirect

Seizure

Table 1
Type and frequencies of reported prodromal symptoms (PS) in the study population.

Type of PS	*N*
Behavioral changes	13
Cognitive disturbances	11
Anxiety and mood changes	9
Fatigue	7
Sleep disturbances	7
Dysthermia	6
Speech disturbances	4
Voiding changes	4
Gastrointestinal symptoms	3
Headache	2
Changes in appetite	2
Faresthesias	1
Pain (other than headache)	1
Other	5
Total	75

Prodromal symptoms were reported to have an insidious onset and their duration ranged from 30 min to several hours. The potential value of prodromes in seizure anticipation would allow the use of preventive and therapeutic measures, including drugs, neurostimulation procedures and behavioral intervention.
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Case 2

- Male, 49 years old. Right-handed.
- No family history.
- No relevant PMH. Anxiety and depressive symptoms.
- Epilepsy onset: 12 yo.
- Focal seizures, starting with a perception of depersonalization ("as if he was in a movie"), followed by impaired awareness. 1-4/year
- Sometimes he refers either dysmnesic and/or epigastric auras.
- Sporadic bilateral tonic-clonic seizures.
- Normal imaging and EEG with left FT spikes.
- Non-adherence to treatment and lost to follow-up.

Case 2 His prodromes

- Before bilateral tonic-clonic seizures:
  - progressive fatigue and tiredness, during 24 hours preceding his last GTCS
  - Speech disturbance: difficulty moving the tongue, changing words, starting a few hours before the seizure and lasting up to few hours afterwards.
- Before focal seizures
  - "I have the feeling of slow thinking and I have to think everything twice; I can´t remember what I have to do. It seems as if my mind were in slow motion." Starts hours, sometimes a whole day before the seizure, and may last up to a few hours after the seizure end.

Your hypothesis??

Epilepsy & Behavior 21 (2011) 184–188

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Are prodromes preictal events? A prospective PDA-based study

Thomas Maiwald [a,b], Julie Blumberg [c,d], Jens Timmer [a], Andreas Schulze-Bonhage [b,*]

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[b] Systems Biology Department, Harvard Medical School, Boston MA, USA
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Keywords:
Prodrome
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Prediction
Epilepsy
Personal digital assistant
Ambulatory monitoring
Prospective

ABSTRACT

Up to 29% of patients with epilepsy report "prodromal" sensations more than 30 minutes prior to seizures. We developed and implemented an objective methodology to prospectively assess the sensitivity and specificity of these subjective experiences using personal digital assistants (*PDAs*). The key property, in contrast to paper-based diaries, is the internal recording of the patient's entering time of prodromes and seizures. Of 500 patients with epilepsy interviewed, 31 claimed to sense prodromal symptoms at least 30 minutes before seizure onset. Eleven of them agreed to participate in a 4-week study to objectively measure their prospective prediction performance. In 9 patients returning data, the majority of prodrome entries were not followed by seizures or were identified only retrospectively. Statistical analysis revealed that no patient could outperform a nonspecific random predictor when predicting seizures based on the occurrence of prodromes, and that the group performance matched precisely the expected result for a by-chance prediction. These results question the predictive value of "prodromes" and the specificity of their occurrence in the preictal period.

Are prodromes preictal events? A prospective PDA-based study

Thomas Maiwald [a,b], Julie Blumberg [c,d], Jens Timmer [a], Andreas Schulze-Bonhage [b,*]

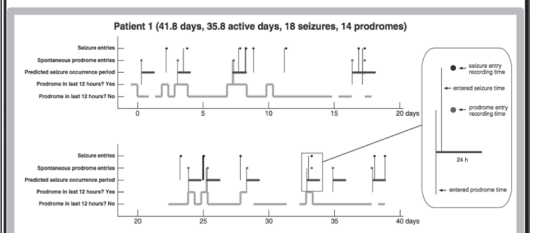

Fig. 2. Overview of events and patient compliance. Patient 1 participated for 42 days and had 18 seizures and 14 prodromes. The upper row represents the experienced seizures (black vertical lines) and their recording times (black dots). Similarly, the middle row shows the stored prodromes (red vertical lines) and their recording times (red dots). Each prodrome is followed by a 24-hour seizure SOP (blue horizontal lines). If a seizure occurs within a SOP, it is classified as correctly predicted by the corresponding prodrome for a SOP of 24 hours. The lower rows display the answers of the triggered prodrome entries. Upper levels of the light blue line belong to periods in which one or more prodromes should have occurred if the patient made consistent entries. The line is interrupted if no answer is given by the patient for a triggered event.

Seizure 22 (2013) 522–527

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Seizure

journal homepage: www.elsevier.com/locate/yseiz

Epileptic prodromes: Are they nonconvulsive status epilepticus?

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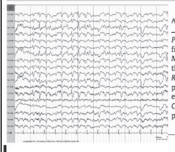


ABSTRACT

Purpose: The aim of this study was to assess how frequently prodromes occur in an adult patient group from a tertiary referral epilepsy centre and to investigate the EEG changes during the prodromes.
Methods: 578 consecutive patients were interviewed on subjective phenomena, experiences heralding the seizures, for at least 30 min before the start of the seizure. EEGs were recorded during the prodromes.
Results: Ten out of 490 included patients had prodromes (2%). We were able to record EEG during prodromes in 6 patients. Three patients had EEG changes corresponding to nonconvulsive status epilepticus. Three patients had unrevealing EEG recordings during prodromes.
Conclusion: Our results suggest that at least in a part of the patients, the prodromes are actually ictal phenomena, and should be treated as nonconvulsive status epilepticus.

Pre-ictal phase

PRECIPITATING FACTORS

PRECIPITATING FACTORS and REFLEX SEIZURES

PRECIPITATING FACTOR:
In most of the cases seizure occurs spontaneously, but there may be association with various triggers. These triggers may act as seizure precipitating factors.

REFLEX SEIZURE: Objectively and consistently demonstrated to be evoked by a specific afferent stimulus or by activity of the patient. (Blume WT et al., 2001)

- Specificity of stim/activity
- Internal /external
- Simple / Elaborated
- Time lapse
- Event (specific or non-specific sz type or trait)

Case 3

- Female, 15 yo. Right-handed.
- PMH unremarkable. No family history.
- Focal epilepsy of unknown etiology
- Onset 13 yo
- Focal aware seizures: olfactory (unpleasant, strong perception) followed by epigastric aura, eventually presenting a vertiginous sensation and impairment of awareness with automatisms.
- Sporadic bilateral TC seizures
- Precipitating factors: stress, sleep deprivation

Case 3

- After some years, she noticed that:

1. Once she perceives the strong, unpleasant and unexplained odor, she can stop the seizure progression by inhaling parfum.
2. A certain odor (bath soap) could precipitate her seizures.
3. When she perceives the precipitating odor, she can sometimes avoid the seizure by inhaling parfum.

Case 3

Hypothesis?

- For clinical approach
- Any research hypothesis?

Hypothesis

- **Clinical hypothesis**
  - Epileptogenic zone in a "non-lesional" patient
  - Fitting clinical hypothesis with a structural lesion
- **Research hypothesis?**
  - Network-etiology-epilepsy type specificity of PF/RS?
  - Tailored non-pharmacological treatment in patients with reflex seizures?

Post-surgical period

PERCEPTUAL CHANGES

Case 1

- Male, 30 yo
- Epilepsy onset 19 yo. Refractory.
- **Right MTS**
- Atypical features: reflex seizures (cognitive task)
- AEDs at time of surgery: CBZ + LTG
- Surgery (2010): selective amigdalo-hippocampectomy
- **Perceptual disturbance modality: olfactory.**

Referred enhanced perception of odors "could not avoid perceiving even those fragrances that are usually on the background and are not consciously perceived." No changes in the perception quality or emotional reaction to it.

- Duration: 3 months

- Simultaneous decrease in libido, during the same time window
- Emotional instability early after surgery
- Seizure free first year after surgery; late relapse with different seizure type.

Case 2

- Male, 39 yo
- Epilepsy onset 18 yo. Refractory
- **Right MTS**
- Atypical features: early bilateral spread on ictal EEG
- AEDs at time of surgery: PHT + VPA + CLZ
- Surgery (2010): Anterior temporal lobectomy
- **Perceptual disturbance modality: olfactory.**

"All odors perceived strongly and are felt with disgust, associating rejection of food intake and sexual approach".

- Duration: 3 months

- Simultaneous decrease in libido and erectile dysfunction, persisting with some fluctuations along the following years
- Late depression (1 yr after surgery)
- Seizure free since surgery

Case 3

- Female, 39 yo.
- Epilepsy onset 15 yo
- Left MTLE. Refractory
- **Low grade tumor; Left amygdala**
- AEDs at time of surgery: CBZ
- Surgery (2013): Lesionectomy
- **Perceptual disturbance modality: auditory**

"Used to like music at very high volume before surgery; afterwards, she prefers it at a much lower volume"

Duration: permanent /long lasting

- No sexual or behavioral changes
- Mood instability (alternating euphoria, anxiety, depression, irritability) during the whole first year after surgery (improvement under SSRI and decreasing CBZ)
- Seizure free since surgery

Case 4

- Female, 36 yo
- Epilepsy onset 7 yo. Refractory
- **Right MTS**
- Atypical features: ictal semiology (hot facial sensation associated to epigastric aura)
- AEDs at time of surgery: LTG + CLZ
- Surgery (2013): Anterior temporal lobectomy
- **Perceptual disturbance modality: visual**

"Hypersensitivity to light, perceived as excessively bright", "glare"

Duration: 2 - 3 months

- No visual field deficit identified
- Simultaneous decrease in libido (perceived as "normalization" by the patient); anxiety, irritability, some phobic behavior. All improving by 3 months.
- Seizure free during 6 months; isolated auras afterwards.

Case 5

Male, 37 yo

Epilepsy onset 10 yo. Refractory

Nodular periventricuar heterotopia Right temporo-occipital region

AED at time of surgeries: CBZ

Surgery 1 (2011): subtotal resection

No visual field deficits

Perceptual disturbance modality: visual

"moving vertical line superimposed in the normal visual field; while looking upward: line + small circle on the top"

Duration: 2 months

No sexual, mood or behavior changes recognized.

- Seizure recurrence after 6 months; depression.

Surgery 2 (2013): total resection

Left homonymus hemianopia

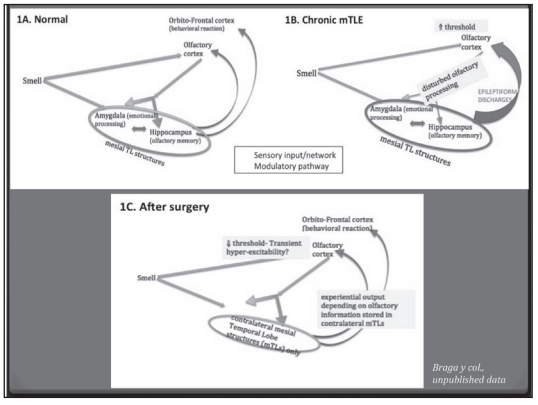
Perceptual disturbance modality: visual

Hypersensitivity to light, either natural or artificial "glare"

Duration 5-6 months

Simultaneous depression; improved afterwards, SSRI.

No sexual dysfunction identified.



- In patients with refractory MTLE, frequent epileptiform discharges arising from amygdalo-hippocampal structures, may generate an adaptive response with increased threshold of the olfactory cortex.
- After surgery, enhanced olfactory perception present in **Cases 1-2**, could be related to a release phenomena at olfactory cortex level.
- The modification of the emotional reaction to odors could be explained by the removal of the Right hippocampus, main center of olfactory memory.

In **Case 3** frequent epileptiform discharges arising from amygdala may have determined an increased threshold of the auditory cortex, which reversed after surgery.

Visual disturbances referred in **Cases 4 and 5** may have a similar explanation, through a transient hyperexcitability of the visual cortex after surgery, although a direct lesion to the visual pathway can not be excluded, leading to deafferentation phenomena.

Perceptual disturbances after epilepsy surgery

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Epilepsy Section, Institute of Neurology. Hospital de Clínicas, Facultad de Medicina, Universidad de la República. Montevideo, Uruguay

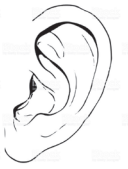
CONCLUSIONS

- We propose the hypothesis that compensatory mechanisms involving changes of excitability in functional networks connected to epileptic foci (increased threshold with inhibitory effect), and their reset after surgery (decreasing threshold to normal parameters, with transient relative hyper-excitability), may underlie these postoperative perceptual changes.
- Disruption of limbic circuits by surgery allow for plastic changes in connectivity and excitability, with potential impact on emotion, behavior and perceptual processing.
- Perceptual, as well as behavioral, sexual and mood changes, should be addressed in patients after epilepsy surgery.
- Further research is needed in order to identify risk factors, outcome and underlying physiopathology.

Poster presentation, IEC 2015, Istanbul (unpublished data)

Final remarks

- Advanced technology is extremely helpful for analyzing and demonstrating neural connectivity.
- Clinical descriptions are still useful both to choose relevant questions in clinical practice, and to foster new answers.
- If you are a clinician, and particularly if you are or want to be an epileptologist, do never forget to use this fantastic tool



- And please remember, when questioning on our brain functioning, to ask the proper question to the adequate interlocutor: your own brain!!

Thank you for your attention!!!

Imad Najm (USA), Patricia Braga (Uruguay)

MAPPING EPILEPTIC NETWORKS (CASE DISCUSSIONS)

Elza Marcia Yacubian (Brazil)

EEG AS BASIS FOR UNDERSTANDING CONNECTIVITY IN EPILEPSIES

EEG COMO BASE PARA ENTENDER LA CONECTIVIDAD EN EPILEPSIAS

Elza Márcia Yacubian
Unidade de Pesquisa e Tratamento das Epilepsias
UNIFESP

Introducción

- La localización del área epileptogénica es fundamental en la evaluación de la epilepsia;
- Hay muchas técnicas de localización no invasivas de redes eléctricas y conectividad como RM, PET, SPECT, RMf, MEG, EEG;
- Sólo EEG y MEG son mediciones directas del funcionamiento cerebral;
- PET, SPECT y RMf son medidas secundarias de la función cerebral, pues evalúan el metabolismo/flujo sanguíneo cerebral.

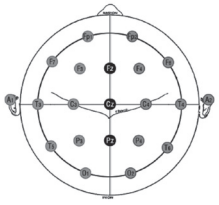
Introducción

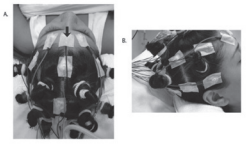
- Sólo EEG y MEG son:
  - Métodos directos de evaluación de la patofisiología en la epilepsia;
  - Hechos en tiempo real con resolución en mseg;
  - Proporcionan la secuencia temporal de la actividad con el fin de permitir la evaluación de la propagación.

BIOFÍSICA DE LOS CAMPOS EEG Y MEG

ELECTROENCEFALOGRAMA

El sistema 10-20 de colocación de electrodos

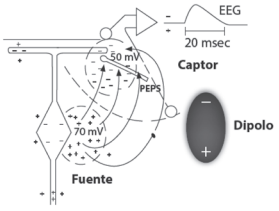




21 electrodos

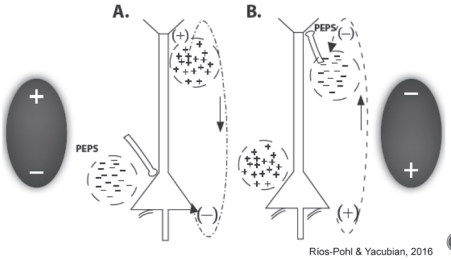
Ríos-Pohl & Yacubian, 2016

Electrogénesis



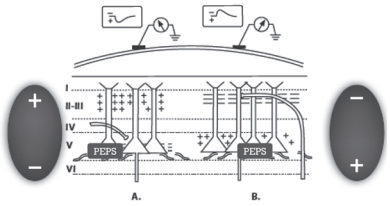
Ríos-Pohl & Yacubian, 2016

Electrogénesis



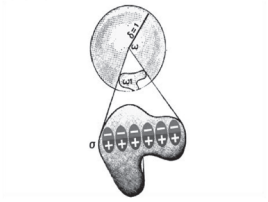
Ríos-Pohl & Yacubian, 2016

Electrogénesis



Ríos-Pohl & Yacubian, 2016

Electrogénesis

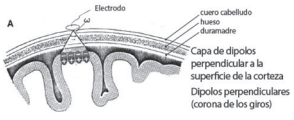


Teorema del ángulo sólido de Woodbury

Ríos-Pohl & Yacubian, 2016

CAMPOS RADIALES Y TANGENCIALES

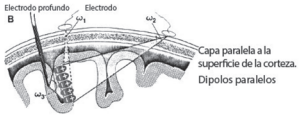
- Radiales- orientación de las células piramidales y de sus campos son ortogonal al cráneo (convexidad cortical). EEG es más sensible, MEG no es sensible;



Ríos-Pohl & Yacubian, 2016

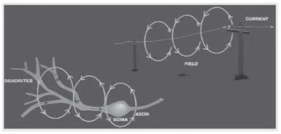
CAMPOS RADIALES Y TANGENCIALES

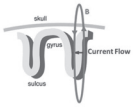
- Tangenciales- orientación de las células piramidales y de sus campos son paralelos al cráneo (por ejemplo, surcos o fisuras profundas). EEG es menos sensible que en la evaluación de los dipolos radiales.

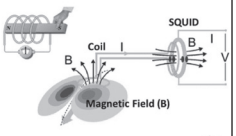


Ríos-Pohl & Yacubian, 2016

¿Por qué MEG?



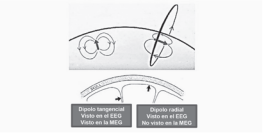




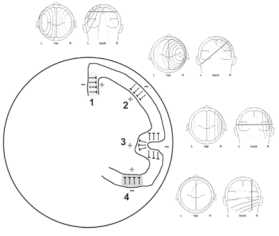
¿Por qué MEG?

Corrientes radiales no producirán campos magnéticos fuera de la cabeza

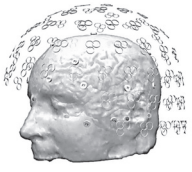
MEG detecta sólo las **corrientes tangenciales**

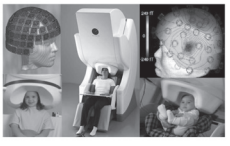


CAMPOS RADIALES Y TANGENCIALES



Magnetoencefalografía
200- > 300 sensores



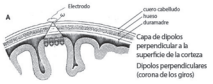


- La señal de MEG decae más rápidamente con la distancia que el del EEG-difícil el registro de estructuras profundas

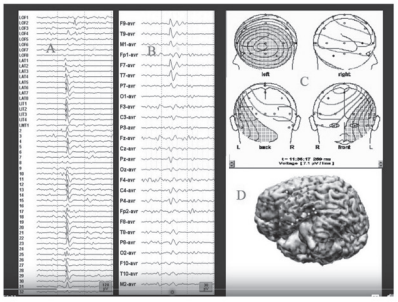
- El hueso es transparente al magnetismo y los campos magnéticos no sufren la resistencia del cráneo

Sensibilidad del EEG

- Requiere > 6 cm^2 de corteza sincronizada;
- Visualiza generadores en los surcos y fisuras pero menos en generadores de los giros;
- EEG es sensible a todas las orientaciones de los generadores, pero de los radiales más que de las tangenciales.



Ríos-Pohl & Yacubian, 2016



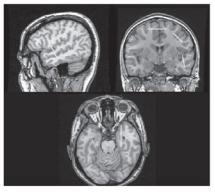
Ventajas del EEG

- Sensible a todas las orientaciones de los generadores;
- Caracteriza la extensión de la propagación;
- Permite registros prolongados, aumentando la posibilidad del registro de crisis epilépticas;
- Es parte de la evaluación de rutina en epilepsia;
- Relativamente barato.

Sensibilidad de la MEG

- MEG requiere 4-6 cm^2 de corteza sincronizada;
- MEG visualiza surcos grandes, fisuras y planos tangenciales, pero no la corteza de la convexidad;
- Los dipolos de la MEG reflejan de forma más precisa la ubicación del generador;
- Sensible a la orientación de los generadores tangenciales.

Ventajes de la MEG



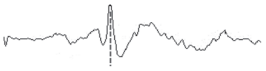
- No es atenuada o distorsionada por el cráneo, los modelos simples de cabeza son adecuados;
- Mayor capacidad de cobertura de la cabeza: 200->300 sensores;
- Sensibilidad superior, ve generadores más pequeños, especialmente los tangenciales a la superficie;
- Precisión superior al EEG en el generador, surcos/giros específicos;
- Sensibilidad a la frecuencia superior (DC a gama).

EEG Y MEG SON COMPLEMENTARES!

ACTIVIDADES EPILEPTIFORMES FOCALES Y GENERALIZADAS EN EL EEG

Descargas epileptiformes focales

Descarga de onda aguda (‘*sharp wave*’)



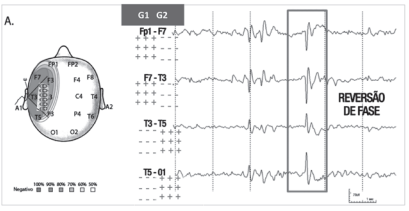
ASIMÉTRICA

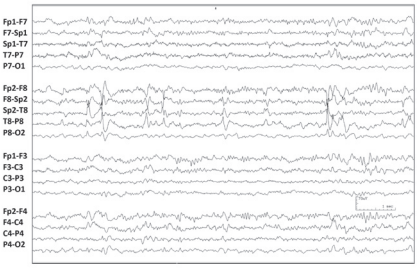
Actividad de superficie negativa, de connotación anormal, epileptiforme, que perturba claramente la actividad de base y compromete dos o más electrodos, cuya duración varía de 70 a 200 mseg. Usualmente la fase ascendente es ligeramente inclinada y la fase descendente aún más inclinada, lo que confiere asimetría al grafoelemento. Es seguida de una onda lenta y su amplitud es variable. Sinónimo de punta lenta (del francés, pointe lente). Sin embargo, se recomienda el término onda aguda (del inglés, sharp wave).

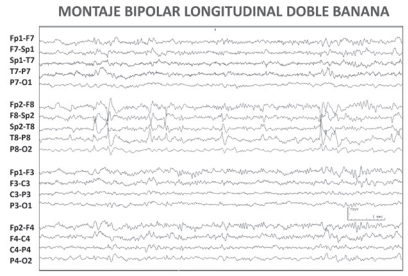
DESCARGAS EPILEPTIFORMES FOCALES

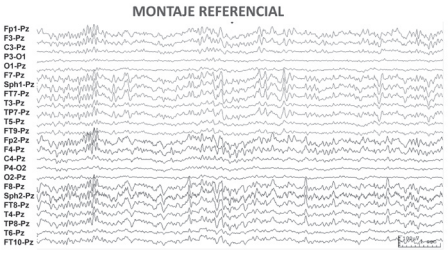
- Pueden ocurrir en cualquier lobo o lado, pero son más comunes en los **lobos temporal y frontal**;
- Son casi siempre de superficie negativa;
- Tiene morfología característica;
- En el montaje bipolar la negatividad máxima es representada por la reversión de fase instrumental.

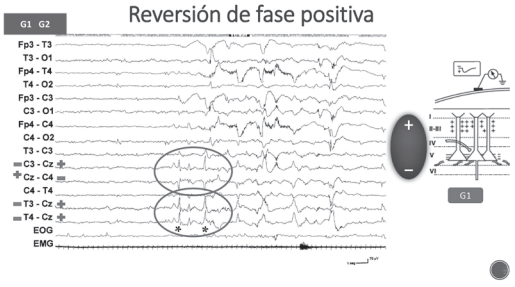
Reversión de fase instrumental
Principio de amplificación diferencial





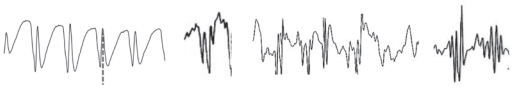






Descargas epileptiformes generalizadas

Descargas de espigas (‘*spikes*’)

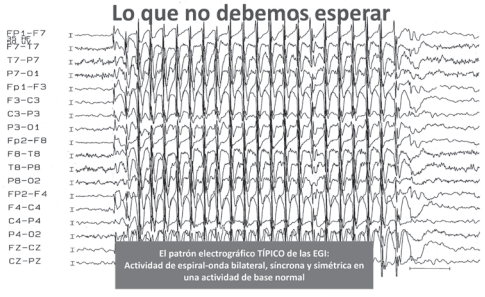


SIMÉTRICA

Actividad de superficie negativa, que claramente se distingue de la actividad de base, que compromete dos o más electrodos, cuya duración es de 20 a 70 mseg. Usualmente sus fases ascendente y descendente son igualmente pronunciadas. Puede o no ser seguida de una onda lenta, pero su forma es relativamente simétrica. Es sinónimo de punta, del francés pointe. Sin embargo, se recomienda el uso del término espiga, del inglés spike.

Penfield W. Brain 1958;81(2):231-4.

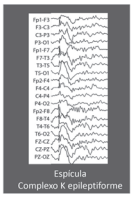
Lo que no debemos esperar



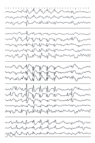
El patrón electrográfico TÍPICO de las EGI:
Actividad de espiral-onda bilateral, síncrona y simétrica en una actividad de base normal

Descargas epileptiformes generalizadas

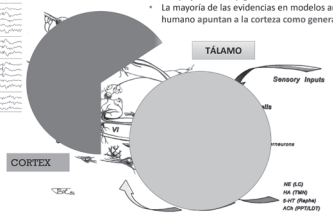
- Usualmente CEO típicos o atípicos o descargas de polispícula, polispícula-onda o ambos;
- 50% pacientes con crisis TCG en el 1º. EEG;
- 1-13% de individuos **sin epilepsia**, particularmente **parientes en primer grado** de pacientes con epilepsias generalizadas.



Espícula
Complexo K epileptiforme



- La corteza y el tálamo se envuelven en la generación de los complejos de la espiga-onda;
- La mayoría de las evidencias en modelos animales y en RMf humano apuntan a la corteza como generador de los CEO.



Blumenfeld. Epilepsia 2005;46 (Suppl 9):S21-S33

Neuropathological Findings in Primary Generalized Epilepsy: A Study of Eight Cases

*H.-J. Meencke and *D. Janz

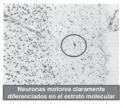
Epilepsia 1984;25(1): 8-21

MICRODISGENESIA o DISPLASIA CORTICAL TIPO 1

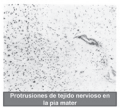
Heinz-Joachim Meencke

Dieter Janz

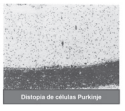
Aumento de las células en el estrato molecular



Neuronas motores claramente diferenciados en el estrato molecular



Protrusiones de tejido nervioso en la pia mater



Distopia de células Purkinje

Dr. Udaya Seneviratne is a Consultant Neurologist and Epileptologist at Monash Medical Centre and St. Vincent's Hospital, Melbourne, Australia

CRITICAL REVIEW AND INVITED COMMENTARY

Focal abnormalities in idiopathic generalized epilepsy: A critical review of the literature

*†Udaya Seneviratne, *Mark Cook, and *Wendyl D'Souza

Epilepsia, 55(8):1577–1588, 2014
doi: 10.1111/epi.12688

- Cambios focales en el EEG van del 16% al 56% en 12 publicaciones;
- Una señal de peor pronóstico en epilepsias generalizadas genéticas.

¿Qué significan descargas focales, unilaterales o asimétricas en las EGI?

- Heterogeneidad amplia entre los estudios;
- Prevalencia de estas "anormalidades atípicas" es difícil de estimar;
- Las anormalidades atípicas en el EEG pueden ser responsables de **errores diagnósticos y elección de FAE inadecuados en EGI**;

Dr. Udaya Seneviratne is a Consultant Neurologist and Epileptologist at Monash Medical Centre and St. Vincent's Hospital, Melbourne, Australia

Clinical Neurophysiology 2016;127(1):214-20.

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Atypical EEG abnormalities in genetic generalized epilepsies

Udaya Seneviratne [a,b,*], Graham Hepworth [c], Mark Cook [a], Wendyl D'Souza [a]

[a] Department of Medicine, St. Vincent's Hospital, The University of Melbourne, Melbourne, Australia
[b] Department of Neuroscience, Monash Medical Centre, Melbourne, Australia

EDITORIAL
GENERALIZED EPILEPSY:
Dont't look too close...
Leijten FSS & Zijlman M (Clinical Neurophysiology 2015)

5. Descargas focales (confinadas a un sólo lóbo o parte de un lóbo);
4. Descargas bifrontales (simétricas y asimétricas) se consideraron fragmentos de expresión frontal de actividad epileptiforme generalizada y no se clasificaron como focales.

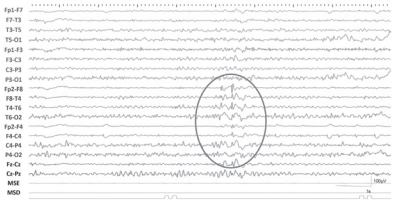
Cinco características atípicas de los paroxismos

1. Inicio focal de los paroxismos;
2. Asimetría de amplitud de los paroxismos y fragmentos;
3. Término focal de los paroxismos;
4. Morfología atípica de los paroxismos;
5. Ritmo rápido paroxístico generalizado.

Seneviratne et al. Clinical Neurophysiol 2016;127(1):214-20

1. Anormalidades focales en el EEG (EMJ)

Mujer, 18 años, 1ª. crisis: 9 años. EMJ + inducción por praxia. Monoterapia VPA. Crisis mioclónicas frecuentes. Crisis TCG controladas.



Dos descargas fronto-centrales focales. Observe la reversión de fase negativa en F4-C4.

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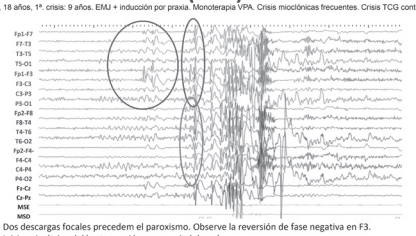
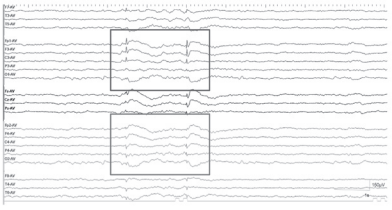


Dos descargas focales precedem el paroxismo. Observe la reversión de fase negativa en F3.
Inicio asimétrico del brote> región parassagital derecha.

2. Descargas epileptiformes asimétricas (EMJ)

Hombre, 23 años, inicio de la epilepsia 20 años, EMJ. Fenobarbital. Sin crisis hace un año.



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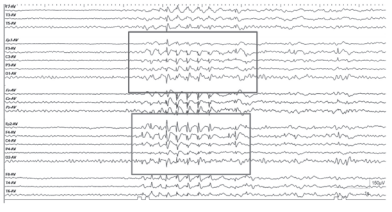
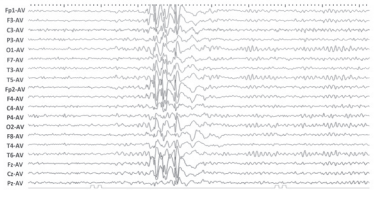


3. Morfologia atípica

Epilepsia ausencia juvenil

Mujer, 50 años, inicio de las crisis a los 7 años. EAJ refractaria. Topiramato y fenobarbital. Valproato y etosuximida- efectos adversos. Ausencias relativamente raras. Crisis TCG controladas.



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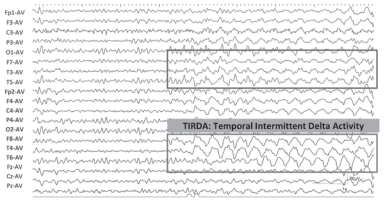

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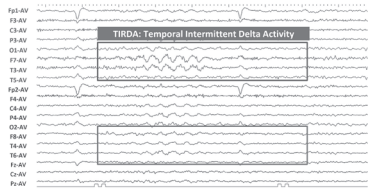
Mujer, 50 años, inicio de las crisis a los 7 años. EAJ refractaria. Topiramato y fenobarbital. Valproato y etosuximida- efectos adversos.

Ausencias relativamente raras. Crisis TCG controladas..

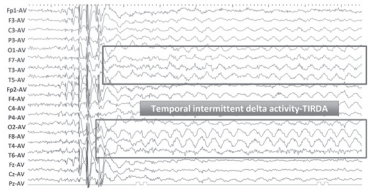


3. Morfologia atípica

Epilepsia ausencia juvenil

Mujer, 50 años, inicio de las crisis a los 7 años. EAJ refractaria. Topiramato y fenobarbital. Valproato y etosuximida- efectos adversos.

Ausencias relativamente raras. Crisis TCG controladas.



¿Qué sabemos sobre TIRDA?

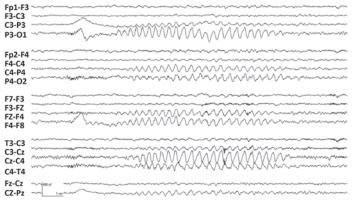
Temporal intermittent rhythmic delta activity (TIRDA) in the diagnosis of complex partial epilepsy: sensitivity, specificity and predictive value.

Reiher J, Beaudry M, Leduc CP. Can J Neurol Sci 1989;16(4):398-401.

- 45/127 registros (35%);
- TIRDA fue más abundante en somnolencia y sueño;
- Cuando ocurre bilateralmente e independientemente, TIRDA varió de lado a lado;
- TIRDA es frecuentemente asociado con **descargas temporales anteriores**, particularmente durante el sueño;
- TIRDA debe considerarse un indicador intermedio de **crisis parciales complejas**.

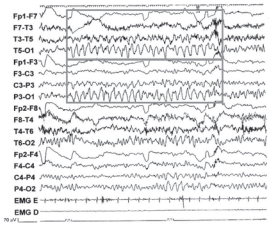
¿Cuál es la actividad delta rítmica más conocida?

ACTIVIDAD DELTA RÍTMICA OCCIPITAL INTERMITENTE
EPILEPSIA AUSENCIA DA INFANCIA

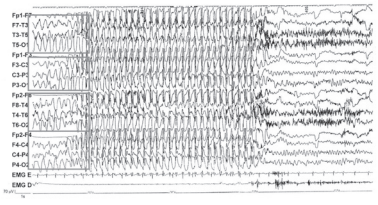


Actividad delta en regiones occipitales, atenuada por la apertura de los ojos y etapas profundas del sueño, acentuada por la hiperventilación y somnolencia- 83% relacionada a las epilepsias generalizadas.

ACTIVIDAD DELTA RÍTMICA OCCIPITAL INTERMITENTE
EPILEPSIA AUSENCIA DA INFANCIA



ATIVIDADE DELTA RÍTMICA OCCIPITAL INTERMITENTE
HIPERVENTILACIÓN



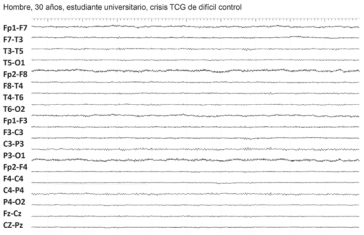
TEMPORAL INTERMITTENT RHYTHMIC DELTA ACTIVITY EN EPILEPSIA AUSENCIA JUVENIL

- Tres casos (13%) en una serie de 23 pacientes con EAJ;
- Nunca observado en 80 pacientes con epilepsia mioclónica juvenil;
- Esta actividad delta fue activada por la hiperventilación y somnolencia. Disminuía en sueño NREM y reapareció en el sueño REM. Su frecuencia era alrededor de 3 Hz;
- TIRDA es muy sugestivo de EAJ así como ondas delta rítmicas posteriores o son de la epilepsia ausencia de la infancia pero con una localización más anterior, sobre los lobos temporales;
- **Este patrón EEG debe ser conocido para evitar el tratamiento de pacientes con EAJ con FAEs inapropiados.**

Gélisse et al. Seizure 2011;20:38-41

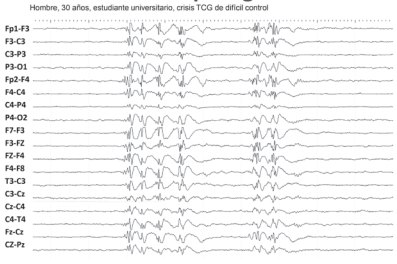
4. Actividad rápida generalizada

Hombre, 30 años, estudiante universitario, crisis TCG de difícil control



4. Actividad rápida generalizada

Hombre, 30 años, estudiante universitario, crisis TCG de difícil control



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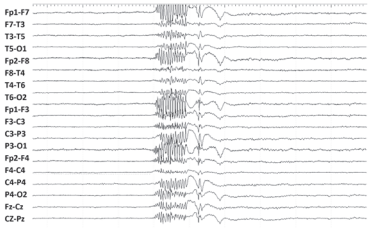

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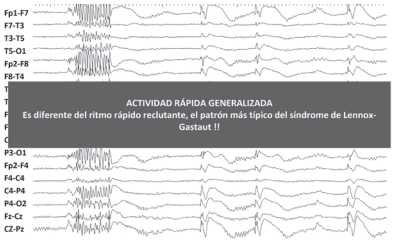


ACTIVIDAD RÁPIDA GENERALIZADA
Es diferente del ritmo rápido reclutante, el patrón más típico del síndrome de Lennox-Gastaut !!

Seneviratne et al. Clinical Neurophysiol 2016;127(1):214-20

Dr. Udaya Seneviratne is a Consultant Neurologist and Epileptologist at Monash Medical University (Clinical), Monash Medical, Melbourne, Australia

Atypical EEG abnormalities in genetic generalized epilepsies

Udaya Seneviratne[a,b,c], Graham Hepworth[c], Mark Cook[a], Wendyl D'Souza[a]

[a]Department of Medicine, St. Vincent's Hospital, Fitz Roy Street, Fitzroy, Melbourne, Victoria, Australia
[b]Department of Medicine, Monash University, Melbourne, Victoria, Australia
[c]Statistical Consulting Centre, The University of Melbourne, Melbourne, Victoria

- Anormalidades epileptiformes atípicas en el 66,4% de 107 pacientes con EGI durante el registro ambulatorio de EEG de 24h;
- Descargas focales en 21,5%;
- Consecuencias de los errores :
  - **Diagnóstico de crisis focales con consecuente tratamiento inefectivo con carbamazepina (exacerbación potencial de crisis de ausencia y mioclónicas);**
  - **Retardo en el diagnóstico (porcentaje de diagnóstico erróneo de EMJ en 5,3% a 91,4% y promedio de retardo en el diagnóstico de 5,9 a 15 años).**

Seneviratne et al. Clinical Neurophysiol 2016;127(1):214-20

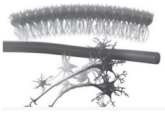
EEG Y RM FUNCIONAL

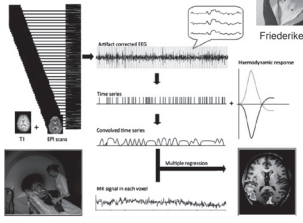
Simultaneous EEG and fMRI recordings (EEG-fMRI) in children with epilepsy

*Friederike Moeller, *Ulrich Stephani, and †Michael Siniatchkin

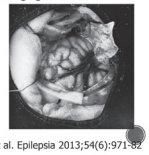
*Department of Neurophysiatrie, Christian-Albrechts-University, Kiel, Germany; and †University Hospital of Child and Adolescent Psychiatry, Johann Wolfgang Goethe-University of Frankfurt, Frankfurt am Main, Germany

Friederike Moeller





Blood Oxygenation Level Dependent Imaging- OxiHMG-DeoxiHMG



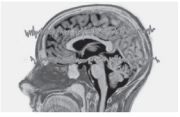
Moeller et al. Epilepsia 2013;54(6):971-82

IMAGEN MULTIMODAL
INFORMACIÓN MORFOFUNCIONAL

- Imágenes adquiridas en tiempos diferentes que se basan en las técnicas de manipulación de imágenes digitales.
- Imágenes adquiridas simultaneamente (síncronas), las cuales son unidas automáticamente.

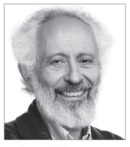
¿Cuándo?

EEG-MEG
Buena resolución temporal;
Baja resolución espacial;
Limitación para la detección de estructuras profundas;
Corrección de los generadores.



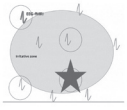
¿Dónde?

RM funcional
Alta resolución espacial;
Sensible a las señales profundas y superficiales;
Alteraciones hemodinámicas cerebrales.

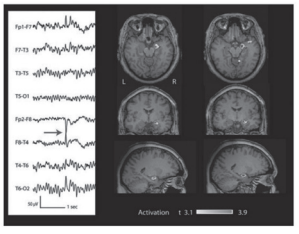


Jean Gotman

Spike onset zone as the Seizure onset zone



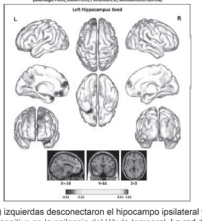
EEG- fMRI- Estudios interictales en epilepsias focales



Epilepsia

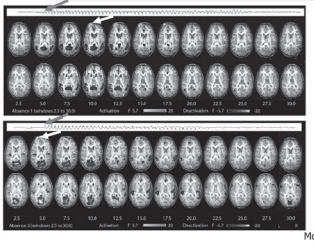
Real-time effects of interictal spikes on hippocampus and amygdala functional connectivity in unilateral temporal lobe epilepsy: An EEG-fMRI study

January 2019



Descargas epileptiformes interictales (DEI) izquierdas desconectaron el hipocampo ipsilateral y la default mode network, que podría estar relacionada con el deterioro cognitivo en la epilepsia del lóbulo temporal. La red de recompensa emocional (más del sistema pre-frontal-límbico) y la red visual también mostraron cambios de conectividad funcional después de DEI izquierdas.

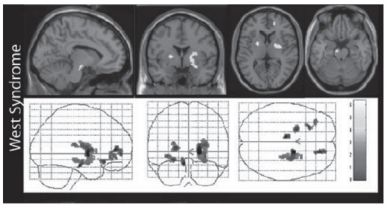
Crisis de ausencias: patrones individuales revelados por EEG-RM funcional



- Activación frontopolar izquierda 10 segundos después del inicio de la ausencia
- Activación talámica 7,5 s tras el inicio de la ausencia en ambas
- Activación frontopolar izquierda 5 s tras el inicio de la ausencia

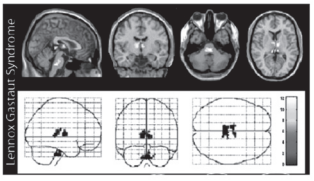
Moeller et al. Epilepsia 2013;54(6):971-82

EEG- fMRI
Encefalopatias epilépticas- Síndrome de West



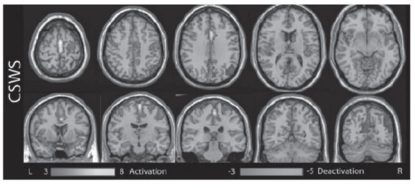
Moeller et al. Epilepsia 2013;54(6):971-82

EEG- fMRI
Encefalopatías epilépticas- Síndrome de Lennox-Gastaut



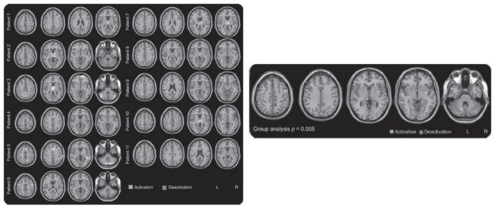
Moeller et al. Epilepsia 2013;54(6):971-82

EEG- fMRI
Encefalopatías epilépticas- Punta-onda continua en sueño



Moeller et al. Epilepsia 2013;54(6):971-82

EEG- fMRI
Síndrome de Doose



Moeller et al. Neurology 2014;82(17):1508-13

Pero esto quien nos dirá en detalles es la Dra.
Clarissa Yasuda

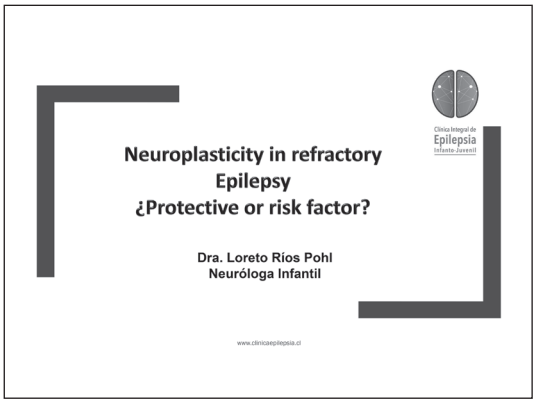
MUCHAS GRACIAS!

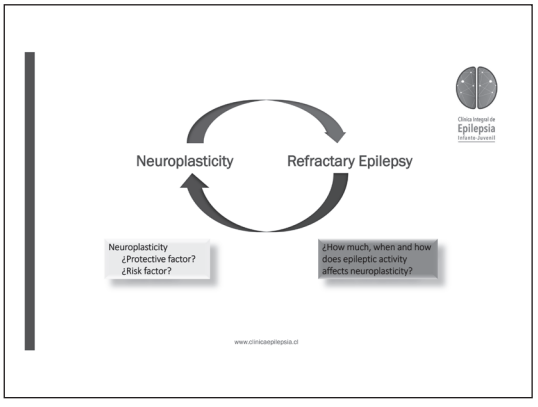
Clarissa Lin Yasuda (Brazil)

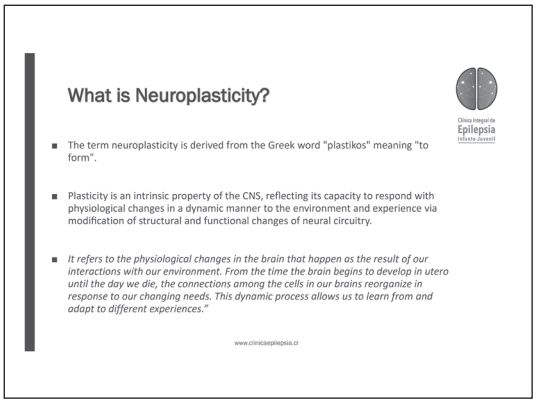
BRAIN CONNECTIVITY IN GENERALIZED EPILEPSIES

Loreto Rios (Chile)

NEUROPLASTICITY IN REFRACTORY EPILEPSY: IS IT A PROTECTIVE OR RISK FACTOR?







- Different as computers, which are built to certain specifications and receive software updates periodically, our brains can actually receive hardware updates in addition to software updates. Different pathways form and fall dormant, are created and are discarded, according to our experiences.

- When we learn something new, we create new connections between our neurons. We rewire our brains to adapt to new circumstances. This happens on a daily basis, but it's also something that we can encourage and stimulate.

www.clinicaepilepsia.cl

Neuroplasticity has 3 particular characteristics:

1. It happens at any time of life.

2. Presents a CRITICAL PERIOD of maximum efficiency and effectiveness.

"Immature Brain"

3. It implies long lasting changes of Learning and Memory.

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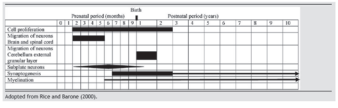
Brain maturation is not linear:

- It is punctuated by a series of developmental spurts, some additive and some regressive, consisting in stepwise processes with 'critical' or 'sensitive' periods.

- This critical periods mark phases of increased plasticity where specific brain circuits are maximally sensitive to acquiring certain kinds of information, or even need that information to be consolidated so that the system involved can establish interconnections with other system.



Adopted from Rice and Barone (2000).

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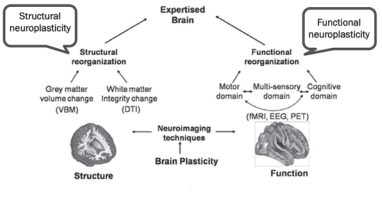
Many neuroscientists use the word neuroplasticity as an umbrella term, it means different things to researchers in different subfields...



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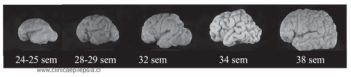
Structural Neuroplasticity

- During human brain development through infancy and childhood, microstructural and macrostructural changes take place to reshape the brain's structural networks and better adapt them to sophisticated functional and cognitive requirements.
- At born the brain has 100 billion brain cell and 15,000 synaptic connections for each one of them.
- However, the development of the brain at Newborn time is incredibly inconclusive. Most neurons are not connected in networks yet.
- At birth, the RN has only **26%** of the volume it will reach at maturity.



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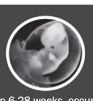
Clínica Integral de Epilepsia Infanto-Juvenil

PRENATAL AND PERINATAL PERIOD

Dynamic and extremely organized processes occurs based on a series of precise and genetically time stablished predetermined but overlapping stages.



- Between 6-28 weeks, occurs neuroblast proliferation and migration allowing

"Overproduction of neurons as storage"

- Once migrations has occurred, starts the differentiation process were specific cells populations emerge in particular regions and become committed to develop into specific CNS structures.

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Clínica Integral de Epilepsia Infanto-Juvenil

PRENATAL AND PERINATAL PERIOD

Dynamic and extremely organized processes occurs based on a series of precise and genetically time stablished predetermined but overlapping stages.

Apoptosis

Programmed cell death that eliminates cells with poor or unnecessary synaptic connections

(occurs in nearly half of all neurons during development).

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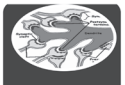
Clínica Integral de Epilepsia Infanto-Juvenil

POST NATAL PERIOD

-This hierarchical period is characterized by differentiation and maturation of CNS progressing into adolescence and early adulthood.

-Growth of short cortico-cortical connections

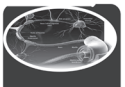
-Myelination and development of local circuitry.



Synaptogenesis

-Rapid and excessive dendritic growth and synaptogenesis with its highest pick around 15 months of age.

-After 2,5 years the initial overproduction of dendrites is followed by pruning, to leave only the most functional branches



Myelination

-This process of insulation ensures rapid transmission of information within and between neural circuits.

-Peaks in myelination have been documented around 2 yrs., 7–9 yrs. and 11–12 yrs.

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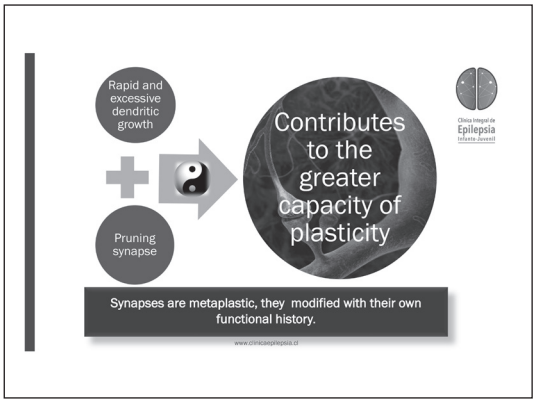
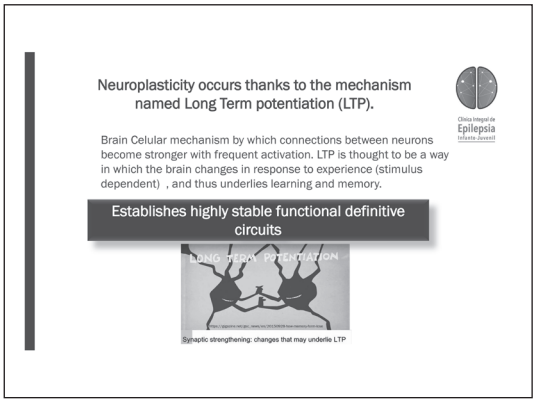
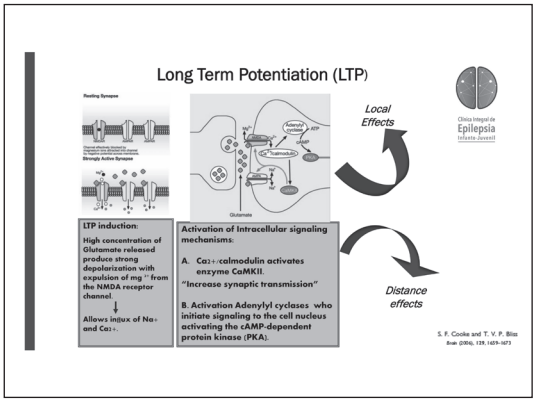
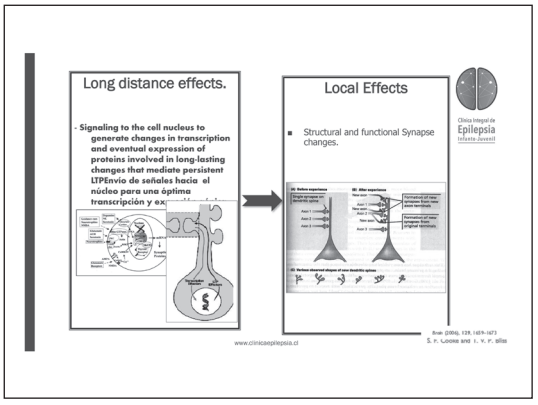
Clínica Integral de Epilepsia Infanto-Juvenil


Rapid and excessive dendritic growth
Pruning synapse
Contributes to the greater capacity of plasticity
Synapses are metaplastic, they modified with their own functional history.
www.clinicaepilepsia.cl


Neuroplasticity occurs thanks to the mechanism named Long Term potentiation (LTP).
Brain Celular mechanism by which connections between neurons become stronger with frequent activation. LTP is thought to be a way in which the brain changes in response to experience (stimulus dependent) , and thus underlies learning and memory.
Establishes highly stable functional definitive circuits
LONG TERM POTENTIATION
Synaptic strengthening: changes that may underlie LTP


Long Term Potentiation (LTP)
Resting Synapse
Strongly Active Synapse
Glutamate
Local Effects
Distance effects
LTP induction:
High concentration of Glutamate released produce strong depolarization with expulsion of mg ++ from the NMDA receptor channel.
Allows influx of Na+ and Ca2+.
Activation of Intracellular signaling mechanisms:
A. Ca2+-calmodulin activates enzyme CaMKII.
"Increase synaptic transmission"
B. Activation Adenylyl cyclases who initiate signaling to the cell nucleus activating the cAMP-dependent protein kinase (PKA).
S. F. Cooke and T. V. P. Bliss
Brain (2006), 129, 1659–1673


Long distance effects.
- Signaling to the cell nucleus to generate changes in transcription and eventual expression of proteins involved in long-lasting changes that mediate persistent LTPEnvio de señales hacia el núcleo para una óptima transcripción y ex
Local Effects
Structural and functional Synapse changes.
Brain (2006), 129, 1659–1673
S. F. Cooke and T. V. P. Bliss
www.clinicaepilepsia.cl

As long Term Potentiation (LTP) exist...

Clínica Integral de Epilepsia Infanto-Juvenil

Long Term Depresion (LTD)
Process by which synaptic connections between neurons are weakened. Important to memory formation-perhaps by resetting previous synaptic changes to allow for new memories to be formed via long-term potentiation (LTP).

Weakening and disappearance of circuits
"Little useful or underutilized"

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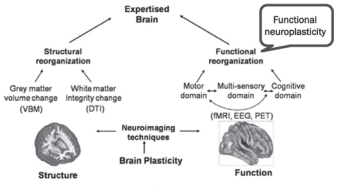

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From Structural to functional plasticity...

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- Until 5 years ago the majority of work in epilepsy was focused on **anatomical changes and pathways** involved in the development of epilepsy (ictogenesis and epileptogenesis).

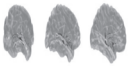

Figure 3 This diagram illustrates anatomical pathways of possible seizure spread in patients with TLE. The lines represent principal anatomical connectivity routes connecting the hippocampus and amygdala to frontal, orbitofrontal and cingulate regions (green), medial temporal and occipital regions (cyan), insula and subcortical grey matter structures (blue), lateral and polar temporal regions (yellow) and contralateral medial temporal structure (black).

Quant Imaging Med Surg 2015;5(2):204-224

- In last 5 years, there is been an explosion of attention to **functional connectivity.**

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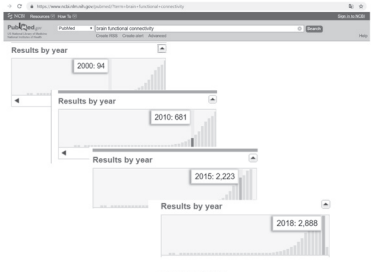

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Functional connectivity
(Functional Neuroplasticity)

Functional refers to the interactions between activities in different brain regions that form a functional connectivity with a resulting network map.

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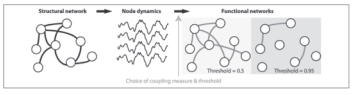
- In neuroscience, "brain networks" (i.e., graphs representing the connectivity of brain components) are typically divided into two categories:
  - *structural networks*
    - the edges represent physical connections between nodes (synaptic or gap junctional connections between individual neurons at microscopic spatial scale)
  - *functional networks*



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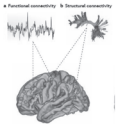
How can we measure different types of connectivity?

- ***Structural connectivity*** *can be measured using diffusion tractography.*
- ***Functional / effective connectivity*** *between nodes are quantified. Both functional connectivity and effective connectivity can be measured during active task performance or task-free ('resting') states using functional MRI.*



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- Neuroplasticity implies stable and permanent structural and functional changes … but "modifiable"

For better or for worse …

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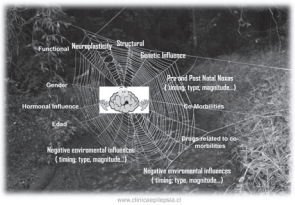
Maximum structural and functional Plasticity / Maximum Vulnerability?



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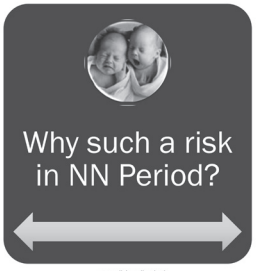

Why such a risk in NN Period?

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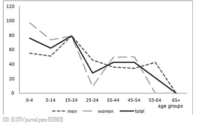
- The neonatal period is the most vulnerable of all periods of life for developing seizures, particularly in the first 1–2 days to the first week from birth.
- The early postnatal development time is a period of increased susceptibility to seizures in relation to other ages.
- This may be due to a combination of factors specific to the developing brain that enhance excitation and diminish inhibition.
- There is an unequal distribution of anticonvulsant and proconvulsant neuro-transmitters and networks



Jensen E. MD. Clin Perinatol. 2010.Dec.;36(4):881

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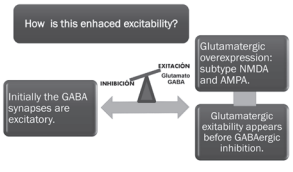
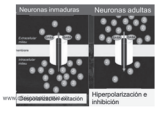
How is this enhaced excitability?





www.despolarización-excitación

Hiperpolarización e inhibición

Lawrence. Semin Pediatr Neurol 17:2010
Ben-Ari, Epileptic Disord 2006;8 (2):

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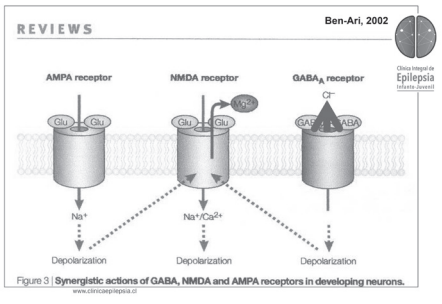
REVIEWS
Ben-Ari, 2002
AMPA receptor
NMDA receptor
GABA$_A$ receptor
Glu
Glu
Glu
Glu
Vgcc
Cl⁻
GABA
GABA
Na⁺
Na⁺/Ca²⁺
Depolarization
Depolarization
Depolarization
Figure 3 | Synergistic actions of GABA, NMDA and AMPA receptors in developing neurons.
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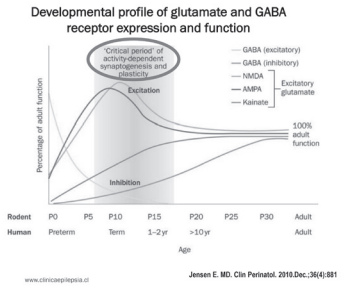
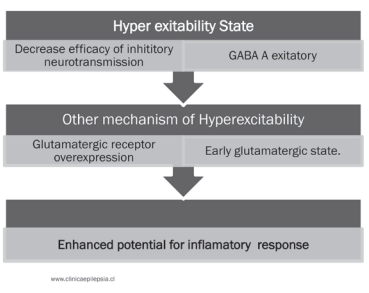
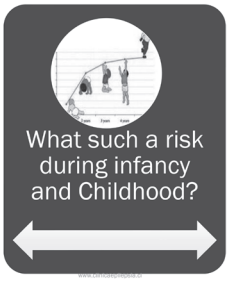

Developmental profile of glutamate and GABA receptor expression and function
'Critical period' of activity-dependent synaptogenesis and plasticity
Excitation
Inhibition
GABA (excitatory)
GABA (inhibitory)
NMDA
AMPA
Kainate
Excitatory glutamate
100% adult function
Percentage of adult function
Rodent P0 P5 P10 P15 P20 P25 P30 Adult
Human Preterm Term 1–2 yr >10 yr Adult
Age
www.clinicaepilepsia.cl
Jensen E. MD. Clin Perinatol. 2010.Dec.;36(4):881


Hyper exitability State
Decrease efficacy of inhititory neurotransmission
GABA A exitatory
Other mechanism of Hyperexcitability
Glutamatergic receptor overexpression
Early glutamatergic state.
Enhanced potential for inflamatory response
www.clinicaepilepsia.cl


What such a risk during infancy and Childhood?

Excesive Synaptogenic , Pruning and myelination.

- This critical period where exists an excessive synaptogenesis and secondary pruning allows that internal and external experiences can have its peak effect on development or learning.
- But this excessive synaptogenic and prune capacity can become the brain´s Achilles heel in situations when excitatory mechanism become over stimulated resulting in maladaptive neuronal circuits resulting in an "Epileptic Brain"

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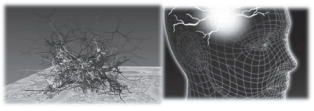
- This "epileptic brain" results of a combination between:
  - *"Useful" neuronal loss.*
  - *Increase in excitability.*
  - *Formation of anomalous circuits with recruitment of peripheral circuits, revealing complex propagations, even to non-continuous areas.*



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- A generic property of any network is that the dysfunction can spread easily between linked elements, leading to **pathological cascades** that can encompass large swathes of the system.
- It is also possible for focal hyper excitability pathology to disinhibit activity and cause cell death or damage in remote sites owing to excess neuronal stimulation.
- Such excitotoxicity plays a central part in the damage sustained to remote areas following focal cerebral noxas and may underlie the distributed degenerative changes seen in focal epilepsy.

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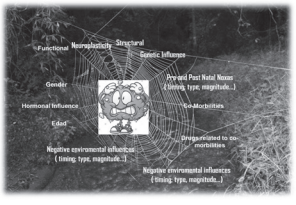
It´s not only the moment, it also matters...



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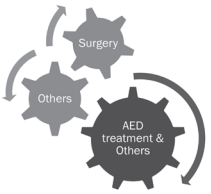
Can we do something for ameliorate this damage?



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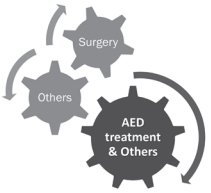
Can we do something for ameliorate this damage?



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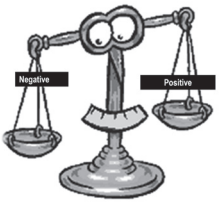
Treating and controlling the Seizures...



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- Studies show that the biggest parents´ problem in a child with epilepsy it is focus on the neurocognitive and behavioral aspects derived from the treatment with AEDs.
- All AEDs can affect in some degree skills as cognitive function, behaviour and global learning.
- Multifactorial influences do these studies with methodological problems, complicating it interpretation.

J.Chil Neurol 2009;24:734-42
Rev. Neurol 2006;43 (supr 1):S 59-70

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Categorical evidences :

- AED's reduction (polytherapy to monotherapy), improves intellectual performance.
- The intake of AED of healthy volunteers induces on them a significant reduction in intellectual performance.
- Patients with recent diagnosis of Epilepsy have a lower cognitive performance after taking a single AED for one month compared to those who didn´t start treatment.

J Child 2004;19 (suppl 1): S15-24
Rev. Neurol 2010;50(Suppl3):S51-6.
Handb Clin Neurol 2013;111:707-18

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- All AED's interact on ion channels, metabolic enzymes, neurotransmitter receptors and transporters:
  - *Modifying discharge properties of neurons.*
  - *Decreasing synchronization.*
  - *Decreasing or Inhibiting propagation.*

Rogawiwski and Löscher, 2004

Controlling Seizures

Neuroplasticity disruption

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This goal of re-establishing the balance "excitation-inhibition":

- It inhibits seizures but ...
- Its not a physiological normalization.
- The physiologically excitatory state in an immature Brain is indispensable for Neuroplasticity.
- Brain Neuroplasticity, defines learning and memory capacity.

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"Early" Surgery

Basically studies before 1997, were focused on seizure control. However, the "current goal" is to improve the cognitive and psychosocial outcome.

- Asarnow et al. Dev Med Child Neurol. 1997
- Looddenkemper et al. Pediatrics 2007

- Cognitive outcome in patients that underwent epilepsy surgery before 2 years old, is better .
- A better previous neurocognitive status is predictor a better outcome.

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Cognitive Function in Preschool Children after Epilepsy Surgery: Rationale for Early Intervention

Hedwig Freitag and Ingrid Tuxhorn

Pediatric Epilepsy Surgery Unit, Bethel Epilepsy Center, Bielefeld, Germany

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CONCLUSIONS

The cognitive performance in pre-surgery patients was directly related to the age of onset of the seizures (<1 year) and extension of the lesion (DC / Tu lobar vs Multilobar / hemispheric)

The duration of epilepsy was the only significant predictor of long-term negative cognitive outcomes.

The early control of the seizures would have a significant impact on the cognitive development potential of children with early onset epilepsy

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Epilepsia, 55(7):1266–1276, 2014
doi: 10.1111/epi.12687, 2014 Epilepsia

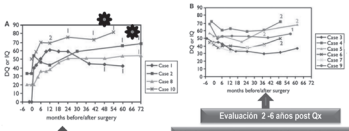
FULL-LENGTH ORIGINAL RESEARCH

Impact of severe epilepsy on development: Recovery potential after successful early epilepsy surgery

*Eliane Roulet-Perez, *Véronique Davidoff, *Claire Meyer-Dubois, †Malin Mander-Ingvar, ‡Margitta Seeck, §Christiane Ruffieux, ¶Jean-Guy Villemure, and *Thierry Deonna

Departments of *Pediatrics and †Neurology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; ‡Department of Neurology, Hôpitaux Universitaires Genève, Geneva, Switzerland; §Institut Universitaire de Médecine Sociale et Préventive, and ¶Department of Neurosurgery, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Niños < 6años operados
Caso 1 al 6 : DC
Caso 7: ET
Caso 8: infarto prenatal
ACM/ESES
Caso 9:Sturge Weber
Caso 10: Tu frontal al



Evaluación 2 -6 años post Qx

Seizure control
"Epileptic activity interruption"

Limited cognitive potential would be given for formation of previous dysfunctional connections, which would limit post-Qx neuroplasticity.

Epilepsia, 47(6):971–980, 2006
doi:10.1111/j.1528-1167.2006.00549.x
© 2006 International League Against Epilepsy

Original Research

Proposed Criteria for Referral and Evaluation of Children for Epilepsy Surgery: Recommendations of the Subcommission for Pediatric Epilepsy Surgery

*J. Helen Cross, †Prasanna Jayakar, ‡Doug Nordli, §Olivier Delalande, ¶Michael Duchowny, ||Heinz G. Wieser, ||Renzo Guerrini, and #Gary W. Mathern

On behalf of the International League Against Epilepsy †Subcommission for Paediatric Epilepsy Surgery, and the Commissions of (Neurosurgery) and (Paediatrics)

Experts have reached the consensus that early surgery is fundamental in patients with "catastrophic" epilepsies in order to prevent regression or development arrest.

At present, the goal of surgery in children is to achieve seizure control, with the potential for the added benefit of improved neurodevelopment. However, favorable seizure outcome after surgery does not guarantee improved behavioral or cognitive status.

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What Surgery?

Based on Structural or functional connectivity??

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There is still a long and complex way to go ...

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GROUP B – CASE STUDY

/ /

Katia Lin (Brazil)

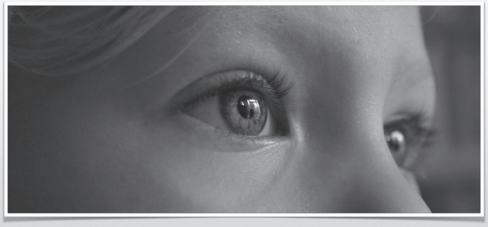
GROUP C – CASE STUDY

GROUP A – CASE STUDY

GROUP D – CONNECTIVITY MODELLING

Guilca Contreras (Venezuela)

CLINICAL CORRELATION OF CONNECTIVITY ALTERATIONS IN CHILDHOOD EPILEPSY WITH CENTROTEMPORAL SPIKES



Epilepsy: from connectivity to connectome

Clinical Correlation of Connectivity Alterations in Children with Childhood Epilepsy with Centrotemporal Spikes

Guilca Contreras

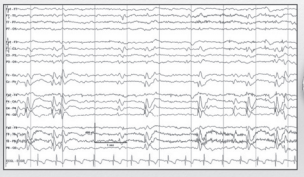
LASSE XIII

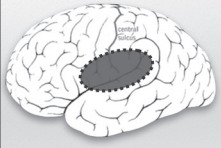
Childhood Epilepsy with Centrotemporal Spikes (CECTS)

- Accounts for 15 – 25% of epilepsy diagnosis in children younger thas 15 years of age [1]. Approximately 700 to 800 new cases of RE are expected each year
- Occurs in neurologically and cognitively healthy children [1]
- Nocturnal focal seizures with a typical EEG that shows centrotemporal spikes (CTs) [1]

1- Engel J, Pedley TA. Epilepsy: a comprehensive textbook. 2nd ed. Philadelphia; 2008.

Childhood Epilepsy with Centrotemporal Spikes (CECTS)





Childhood Epilepsy with Centrotemporal Spikes (CECTS)

- Comorbidity with a wide spectrum of neuropsychological and learning disabilities such as speech and language disorders, reading disabilities, attention impairment, visuomotor and behavior impariments, and psychiatric problems [1-4]
- Attention impairments 67%; language impairments 54%; reading disability 42% [5,6]

CECTS IS NOT BENIGN!!!

1- Deonna T, Zesiger P, Davidoff V, Maurer M, et al. Dev Med Child Neurolog 2000;42:595-603.
2- Goldberg-Stern H, Gouvea OM, Sadeh M, et al. Seizure 2010;19:12-6.
3- Giordani B, Caveney AF, Laughrin D, et al. Epilepsy Res 2006;69:84.
4- Smith AB, Karvos PM, Clarke T, et al. Epilepsia 2012;53:705-711.
5- Smith AB, Bajomo O, Pal DK. Dev Med cjild Neurol 2015;57:1019-1026
6-Demichelis F, Pal DK, McGrowity CJ, et al. Epilepsia 2014;55:90

Childhood Epilepsy with Centrotemporal Spikes (CECTS)

- With its typical onset between the age of 7 - 10 years, RE might critically influence the develompment and maturation of brain networks that are essentially involved in cognitive and psychological functioning
- Several factors such as localization, lateralization, and focality of IEDs may mediate their impact on cognitive development, moreover IEDs may have similar implications on cognition as seizures and might be coupled with the Concept of System Epilepsy[1-4]
- The term "idiopathic"traditionally implies lack of "demonstrable anatomic lesions". However, structural and functional abnormalities have been decribed in typical CECTS children: bilaterally increased gray matter volume in the frontal lobes and insula[5], extensive cortical thinning in frontal, central, parietal and temporal lobes[6]. Abnormal white matter in the frontal and temporal lobes[7]

1- Ritse D, Vago C, Franceschetti S, et al. Epilepsy Behav 2007;10:278-85
2- Wolff M, Weiskopf N, Serra E, et al. Epilepsia 2005;46(10):1661-7
3- Nicolai J, Elves S, Biermans D, et al.Epilepsia 2012;53(6):1051-9
4- Arzenzini G, Mangonotti P, Meletti S, et al. Epilepsia 2012;53(5):771-8
5- Pardoe HR, Berg AT, Archer JS, et al. Epilepsy Res 2013;105:133-9
6- Overvliet GM, Besseling RMH, Jansen JFA, et al. Neuroimage Clin 2013;2
7- Lundberg S, Eeg-Olofsson O, Raininki R, et al. Epilepsia 1999;40:1808-15

Childhood Epilepsy with Centrotemporal Spikes (CECTS)

- Neurocognitive deficit in epilepsy has been considered to be a chronic consequence of repetitive spikes inhibiting the same cortical area over a period of many years, leading to delayed or incomplete maturation of the brain[1-3]

1- Fonseca LC, Tedrus GM, Pacheco EM. Epilepsy Behav 2007;11:65-70
2- Hommet C, Billard C, Motte J, et al. Epileptic Disord 2001;3:207-16
3- Monaijuze C, Broadbent H, Boyd SG, et al. Epilepsia 2011;52:e79-83

Childhood Epilepsy with Centrotemporal Spikes (CECTS)

- Studies of altered functional connetivity in pediatric epilepsy patients are becoming more common, mainly concerning the resting state brain network (rs-fMRI)
- Alterations in the RS FC patterns between specific brain areas would be correlated with cognitive impairments in CECTS
- Language disabilities and academic impairments found in RE are also common in relative of RE probands, with an incidence 5.4 times than in the general population[1]
- The similarity in neurocognitive profiles between probands and siblings, suggest that these neurodevelopmental traits in RE should be genetically influenced[2]

1- Clarke T, strug LJ, Murphy PL, et al. Epilepsia 2007;48(12):2258-65
2- Oliveira EP, Neri ML, Capelatto LL, et al. Arq Neuropsiquiatr 2014;72(11):826-31

Resting functional MRI connectivity studies en patients with CECTS

Authors (year)	BECTS population (mean years ± SD)	Control population (mean years ± SD)	Selected neuropsychology	Analyses	Findings
Jiao et al. (2014)[18]	30 BECTS with IEDs (9 ± 2) 23 BECTS without IEDs (10 ± 2)	28 HC (10 ± 2)	WISC Full-Scale IQ BECTS with IEDs 112 ± 12 BECTS without IEDs 107 ± 15 HC 114 ± 15	Graph theory with regional parcellation; NBS	*Decreased global and local efficiency for both BECTS with and without IEDs; decreased functional connectivity for both BECTS with and without IEDs in L and R FL and R PL*
Luo et al. (2016)[19]	21 BECTS (9 ± 2) (8 subs. excluded)	20 HC (9 ± 2) (3 subs. excluded)	WISC Full-Scale IQ BECTS 81 ± 12 HC 102 ± 6	Seed-based correlations; seed-based GCA	*Increased functional connectivity within and anticorrelation between DMN and TPN; significant difference in outflow/inflow in the left sup. F and L O*
Luo et al. (2015)[20]	21 BECTS (9 ± 2)	20 HC (9 ± 2)	WISC Full-Scale IQ BECTS 78 ± 12 HC 109 ± 6	Seed-based correlations	*Decreased functional connectivity for four regions with increased GMV: R Puta, R Insula/Oper, R SPM and L paracentral lobule*
Wu et al. (2015)[15]	32 BECTS (10 ± 2)	35 HC (10 ± 2)	WISC Full-Scale IQ BECTS 90 ± 8 Controls 100 ± 16	Voxel-mirrored homotopic connectivity	*Decreased interhemispheric connectivity in several FL regions and cerebellum*
Wu et al. (2015)[16]	37 BECTS (10 ± 2)	35 HC (10 ± 2)	WISC Full-Scale IQ BECTS 91 ± 9 Controls 101 ± 16	Seed-based GCA	*Increased driving from sensorimotor cortex to R med FL, pCingG* *Decreased drive to L IPG*
Xiao et al. (2015)[20]	73 BECTS (10 ± 2)	73 HC (10 ± 2)	WISC Full-Scale IQ BECTS 97 ± 13 HC 102 ± 11	Graph theory with regional parcellation; NBS	*Decreased global CC and efficiency; decreased connectivity in sensorimotor regions*
Xiao et al. (2015)[17]	15 BECTS with ADHD (8 ± 2) 15 BECTS without ADHD (9 ± 2)	15 HC (8 ± 2)	WISC Full-Scale IQ BECTS-ADHD 102 ± 6 BECTS w/o ADHD 101 ± 5 HC 102 ± 6	Seed-based correlation	BECTS with ADHD—decreased DAN connectivity BECTS w/o ADHD—increased DAN connectivity BECTS ± ADHD—increased VAN and DMN connectivity
Zeng et al. (2015)[18]	16 new-onset BECTS (9 ± 2) 17 chronic BECTS (11 ± 2)	18 HC (11 ± 2)	Annual Mandarin School Exam New-onset BECTS 81 ± 12 Chronic BECTS 76 ± 13 HC 87 ± 9	ReHo	New-onset BECTS—decreased ReHo in cerebellum, DMPFC, OL; increased ReHo in sensorimotor and language regions Chronic BECTS—decreased ReHo in cerebellum, DMPFC, OL; increased ReHo in language regions
Besseling et al. (2014)[21]	22 BECTS (11 ± 2)	22 HC (11 ± 2)	Nil	Graph theory with regional parcellation; correlation of structural-functional graphs	NAD for connectivity *Decreased structural-functional correlation in medial P and C-T clusters*
Tang et al. (2014)[14]	30 BECTS (10 ± 2)	20 HC (10 ± 2)	WISC Full-Scale IQ BECTS 110 ± 15 HC 116 ± 17	ReHo	*Increased ReHo L IPG, L SPG, L and R preCentG, R postCentG gyrus; R AngG, R SMG, L and R sup P* *Decreased L and R orbito-FL and L and R T poles, R putamen, cerebellum*
Besseling et al. (2013)[12]	22 BECTS (11 ± 2)	22 HC (10 ± 2)	CELF-4 Core Language Score BECTS 95 ± 18 HC 105 ± 11	ICA of task-evoked data	*Decreased connectivity between L IPG and "sensorimotor network"*
Besseling et al. (2013)[13]	23 BECTS (11 ± 2)	21 HC (10 ± 2)	CELF-4 Core Language Score BECTS 92 ± 18 HC 106 ± 11	Seed-based correlation	*Decreased connectivity between L pre- and postCentG and R IPG*

Childhood Epilepsy with Centrotemporal Spikes (CECTS)

- Many researches have searched for a genetic cause of CECTS, but the common form of this epilepsy syndrome itself does not seem to have a clear mendelian inheritance
- Components of the syndrome may be linked more consistently to specific genes. Family association studies have shown that the centrotemporal spike trait if often inherited in an autosomal dominant fashion and may be associated with mutations of the elongator protein complex 4 (ELP4) gene on chromosome 11, but this association has not been consistent[1,2]
- Additional analyses have suggested linkage of speech sound disorder in RE to 11p13 and reading disability to chrosome 7q21 y 1q42. Although RE is a common disorder, the genetics underlying it are complex and still being investigate[2,3]
- **GRIN2A** a gene on chromosome 16p13 that encodes a subunit of the glutamate N-methyl-D aspartate receptor, important in brain development, synaptic plasticity, memory, and sleep. A variety of mutations have been identified en families with ESES, CSWS, LKS (9-20%) and 3.6% of patients with RE[4-6]
- **RFBOX** genes responsible for splicing of neuronal transcripts important in control of membrane excitability[7]

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Review

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Table 1
Main characteristics and results of the studies included.

Study	Participants (n) BC	Participants (n) HC	Study objective	Assessment of language performance	Affected language areas	Influence of clinical factors related to BECTS
Margarian et al., 1995	16	–	Determine if children with BECTS show language disorders, in which areas, and if there is a relationship with the clinical factors related to this condition	Bilan informatif du langage oral à six ans (BILO): expressive grammar and vocabulary (naming; sentence completion); receptive grammar and vocabulary (associations of sentences with pictures); lexical and semantic fluency (deciding connexidness between words and sentences with pictures) Vocabulary Language Test (Malpaquin): receptive and expressive emergent language; speech	Sentence completion Expressive language (unspecified results)	No significant difference between performance of children in the active phases or in remission Right focus associated with worse morphosyntactic abilities
Vinagre et al., 2005	50	–	Characterize the school performance and the neuropsychological profile of children with BECTS, through clinical and electrophysiological data	Vocabulary Language Test (Malpaquin): receptive and expressive language (unspecified results)	Expressive language (unspecified results)	Positive correlation between observed language functions and atypical seizures
Northcott et al., 2005	42	–	Evaluate the effect of interictal EEG activity on the cognitive and behavioral functioning of children with BECTS, and to evaluate the effect of interictal EEG activity	Wide Range Assessment of Memory and Learning (WRAML): verbal memory Clinical Evaluation of Language Fundamentals—3 (CELF-3): receptive and expressive language Peabody Picture Vocabulary Test—3 (PPVT-3): receptive vocabulary Boston Naming Test: expressive vocabulary Queensland University Inventory of Literacy (QIL): phonological awareness Test of Language Competence (TLC): receptive and expressive language Verbal Motor Production Assessment (VMPA): motor speech production	Phonemic manipulation, visual rhyme detection Comprehension of ambiguous sentences Sentence memory	No significant relationship between interictal EEG activity and performance in verbal memory tasks compared to left discharges Spike frequency did not correlate with children's performance.
Margarian et al., 2007	27	54	Assess the language functions of children affected with RE at its remission, to identify the affected areas in the two phases and to analyze the influence of epilepsy focus on linguistic performance	BILO: expressive grammar and vocabulary (naming; sentence completion); receptive grammar and vocabulary (associations of sentences with pictures) Phonology (word repetition) Semantics (pictures naming) Morphosyntactic competence test (PRED-4): (complete sentences using grammatical morphemes) Syntactic-morphosyntactic comprehension (association of sentences with pictures) Lexical and grammatical judgment tasks (deciding concordance between words and sentences with pictures) Prosodic analysis (duration) *gnosem-diligen (PPPC): morphosyntactic functioning* Peabody Pictures Vocabulary Test—3 (PPVT-3): receptive vocabulary Boston Naming Test: expressive vocabulary Test Reception of Grammar: morphosyntactic comprehension Access to mental lexicon: word production by phonemic and semantic categories Wide Range Assessment of Memory and Learning (WRAML): verbal memory Queensland University Inventory of Literacy: phonologic awareness Verbal learning and Memory Test (VLMT): auditory verbal memory Tu Neiger' Luria Christensen Neuropsychological Test Set for Children (TNL): verbal comprehension of real words; expressive and receptive vocabulary Heidelberger Speech Developmental Test—2nd Edition (HSET): expressive and receptive grammar	Sentence completion; sentences-pictures association; use of clitic pronouns Lexical fluency Word repetition Verbal re-elaboration of semantic knowledge Lexical field memory Phonemic fluency Verbal memory Derivation of adjectives Correction of semantically incorrect sentences Analyzes the emotional content of a statement Recognize and express interpersonal relations	Spike focus and age at onset did not influence the language skills Children in remission maintained the difficulties.
Rios et al., 2007	24	18	Ascertain the cognitive and language skills of children with BECTS in the active phase and to evaluate the relationship between some characteristics of the EGG and the explored components	Peabody Pictures Vocabulary Test—3 (PPVT-3): receptive vocabulary Boston Naming Test: expressive vocabulary	Verbal re-elaboration of semantic knowledge	Multifocal location and temporal prominence seem to impair the performance.
Northcott et al., 2007	40	40	Accurately delineate the memory and phonological awareness profile, as well as the quality of life of children with BECTS	Wide Range Assessment of Memory and Learning (WRAML): verbal memory	Phonologic awareness; rhyme detection	–
Vitth-Arenstock et al., 2008	20	20	Assess the language and speech skills of children with BECTS during the active phase of epilepsy and after remission	Queensland University Inventory of Literacy: phonologic awareness Verbal learning and Memory Test (VLMT): auditory verbal memory Tu Neiger' Luria Christensen Neuropsychological Test Set for Children (TNL): verbal comprehension of real words; expressive and receptive vocabulary	Verbal memory Derivation of adjectives Correction of semantically incorrect sentences Analyzes the emotional content of a statement Recognize and express interpersonal relations	No significant influence of AED treatment and localization in the results After remission, language difficulties disappear.

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Goldberg-Stern et al., 2010	36	15	Establish whether the cognitive changes evident in children with BECTS are the result of the number of seizures, the AEDs, or whether it is an inherent characteristic of the syndrome itself	Wechsler Intelligence Scale for Children-Revised (WISC-R): verbal comprehension; vocabulary Verbal fluency: phonetic and semantic word generation Story recall Rey Auditory Verbal Learning Test (RAVLT): repetition of a list of unrelated words	Phonetic verbal fluency Vocabulary	No relationship between laterality, number of AEDs, or seizure frequency with the language competences
Lilipoulos et al., 2009	20	20	Characterize the language profile of children with BECTS, assess language lateralization by means of functional magnetic resonance imaging, and study their relationship	Clinical Evaluation of Language Fundamentals Fourth Edition (CELF—IV): sentence repetition and production	Sentence production	Better performance on sentence production was correlated with increasing left-sided lateralization in the inferior frontal region.
Danielsson et al., 2009	25	50	Assess the cognitive abilities of preschool and elementary school children with BECTS and verify if it is possible to define a cognitive developmental profile of these children	KTT-KIDS: articulation, auditory memory, language comprehension, and word fluency	Language comprehension Articulation Auditory memory	No relationship between clinical factors and children's language abilities No differences in language skills between children with seizures and children with rolandic discharges only Correlation between spike-wave index and language deficits, but not with age of onset, seizure frequency, spike location, and seizure type
Liu et al., 2012	47	30	Evaluate the neuropsychological abilities in children with BECTS and determine whether there is a relationship between clinical factors and cognitive functions	Phonological Awareness Test: onset and rhymes Homophone Production Test: morphological awareness WISC-CN: vocabulary + comprehension	Receptive and expressive vocabulary Rhyme and syllabic segmentation Word production	Younger age at epilepsy onset (under 8Y) had correlation with worse all the results of the language evaluations
JesterDfrow et al., 2012	61	35	Assess language function in children with BECTS and its association with the age of epilepsy onset	Developmental Neuropsychological Assessment Battery (NEPSY): verbal fluency (phonemic and semantic); speeded naming; and comprehension of and instructions of varying syntactic complexity	Phonemic and semantic verbal fluency Naming attributes Comprehension of oral instructions of varying syntactic complexity	No relationship between laterality, number of AEDs, and seizure duration with language function Early onset was associated with worse scores in language tests
Nezi et al., 2012	25	28	Identify changes in executive functions in children with RE and verify the influence of clinical variables	Phonological verbal fluency test (VKS): and semantic fluency test—phonetic and semantic word generation	Phonetic and semantic word generation	Spike frequency and localization in the results After remission, language difficulties disappear.
Filippini et al., 2013	33	–	Analyze the long-term effects of nocturnal interictal epileptiform discharges on neuropsychological Investigate cognitive profiles with RE	Test of Memory and Language: verbal memory; phonemic and semantic verbal fluency Test of Reception Grammar: receptive language Test of Word Finding: lexical retrieval and fluency	Phonological decoding Lexical retrieval	Greater frequency of nocturnal interictal epileptiform discharges, highest number of AEDs and early age of onset was a predictor of worse language outcomes
Overdevest et al., 2013	25	20	Investigate whether abnormalities in cortical thickness can be found in RE and if such abnormalities are localized in classical left perisylvian language areas and are associated with language impairment	Clinical Evaluation of Language Fundamentals (CELF-4): receptive and expressive language (speech production and comprehension); semantic (categorization; word definitions/associations; naming; sentences construction and comprehension); morphosyntactic (sentence comprehension of structural aspects; sentences organization and construction); working and verbal memory (word, sentence and digit repetition and production);	All receptive and of content language tasks Semantic categorization Words definition Auditory comprehension	Age at onset, seizure duration, and spike frequency did not influence the results No associations were found between cortical thickness of perisylvian regions relative to the regions of alternate cortex.
Vannest et al., 2013	17	18	Assess and characterize a possible neurocognitive endophenotype associated with children with RE	MEPD-II: verbal fluency (phonemic and semantic); phonological awareness; comprehension of oral instructions of varying syntactic complexity WISC-IV: verbal comprehension; working memory	Verbal comprehension Short and long-term verbal memory	–
Amaral et al., 2015	13	17	Evaluate temporal auditory processing and phonological awareness in school-age children with BECTS	Phonological Awareness Test (PCF): syllable and phonemic synthesis, segmentation, manipulation and transposition; rhyme; end alliteration.	Rhyme Syllabic segmentation and manipulation Phonemic synthesis and transposition	Correlation between auditory temporal processing and phonological awareness
Maillat et al., 2015	15	18	Examine the reading neuronal networks in children with BECTS	Expressive One Word Picture Vocabulary Test (EOWPVT): naming Children's Verbal Learning Test (CVLT-C): phonological verbal memory Delis-Kaplan Executive Function System Battery: verbal fluency WISC-IV: verbal comprehension	Vocabulary Phonological verbal fluency	Correlation between early epilepsy onset and worse performance in phonological verbal fluency

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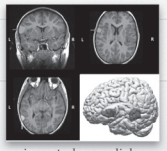

- Since epileptic discharges in this type of epilepsy occur in central or medial regions of the temporal lobe, we consider this a good model to understand the relationship between epileptic activity and language functions
- Semantic, morphosyntactic, and phonological features of the language were identified in the 18 studies
- In the domain of morphosyntax, there is no consensus regardings the altered skills
- As regard the phonological domain, there is some variability among studies, but changes in the intrasyllabic, syllabic, and phonemic levels were identified in tasks of rhyming identification and production, and syllabic and phonemic segmentation and manipulation
- The verbal memoy was also identified as altered

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Role of Language-Related Functional Connectivity in Patients with Benign Childhood Epilepsy with Centrotemporal Spikes

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Chang-hyun Park**
Hye-Sun Hong**
Yun Sae Choi**
Jeong Hyun Yoo*
Hyang Woon Lee**

Table 1. Clinical characteristics of the BECTS patients and healthy controls

Clinical characteristic	BECTS	Controls	p
Number of subjects	19	23	
Sex (male/female)	10:9	9:14	0.382
Age at fMRI study (years)	13.42±2.20	13.17±2.74	0.122
Age at onset (years)	7.3±2.2.1	-	-
Duration of seizure (months)	30.88±7.40.83	-	-
AED at fMRI study			
Duration (months)	67.42±19.26	-	-
Type, n (%)			
None	3 (16.8)		
Carbamazepine	2 (10.5)		
Oxcarbazepine	5 (26.3)		
Valproic acid	5 (26.3)		
Lamotrigine	2 (10.5)		
Dual treatment	2 (10.5)		
EEG epileptiform discharges			
Location, n (%)			
Left CT or CTP	8 (42.1)		
Right CT or CTP	4 (21.1)		
Both CT or CTP	7 (36.8)		
Frequency, n (%)			
None	9 (47.4)		
Rare (≤1 per 10 min)	2 (10.5)		
Occasional (>1 per min)	0		
Frequent (>1 per min)	3 (16.8)		
Abundant (≥1 per 10 sec)	5 (26.3)		

Data are n (%) or mean±standard deviation values.
AED: antiepileptic drug, BECTS: benign childhood epilepsy with centrotemporal spikes, CT: centrotemporal, CTP: centrotemporoparietal, EEG: electroencephalography, fMRI: functional magnetic resonance

Rolandic Mask and Language Area

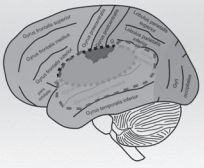

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Table 2. Comparisons of neuropsychological test scores between the BECTS patients and healthy controls

Neuropsychological test	BECTS (n=19)	Controls (n=23)	p
K-WISC-III			
FSIQ	93.37±13.46	118.35±9.52	<0.001*
VIQ	91.79±15.09	119.70±9.89	<0.001*
PIQ	96.53±12.29	112.52±11.24	<0.001*
Absolute V-P	11.16±8.88	10.48±7.99	0.795
Relative V-P	-4.74±13.66	7.26±11.11	0.002*
VCI	92.16±16.75	118.22±10.22	<0.001*
POI	99.89±9.98.87	115.60±13.80	<0.001*
FDI	99.37±18.15	120.00±9.73	<0.001*
Processing speed	89.06±20.08	112.78±15.16	<0.001*
Auditory-verbal memory test			
Immediate recall	9.17±3.37	10.13±2.72	0.516
Delayed recall	9.00±3.36	10.44±1.84	0.147
Recognition	9.67±2.65	9.48±1.99	0.772
Visuospatial memory test			
Copy	15.00±0.68	15.00±1.27	1.000
Immediate recall	11.44±4.20	11.68±2.78	0.832
Delayed recall	11.61±3.03	12.38±3.07	0.438
Executive function test			
Trail-making test part A	32.16±13.39	30.49±8.61	0.650
Trail-making test part B	76.73±43.97	60.49±15.04	0.139
Stroop: word	5.50±3.26	6.64±3.32	0.276
Stroop: color-word	6.33±4.59	7.92±4.19	0.280

Data are mean±standard deviation values.
*Statistical significance at p<0.06.
BECTS: benign childhood epilepsy with centrotemporal spikes, FDI: freedom from distractibility index, FSIQ: full-scale intelligence quotient, K-WISC-III: Korean version of the Wechsler Intelligence Scale for Children-III, PIQ: performance intelligence quotient, POI: perceptual organization index, VCI: verbal comprehension index, VIQ: verbal intelligence quotient, V-P: verbal–performance intelligence quotient discrepancy

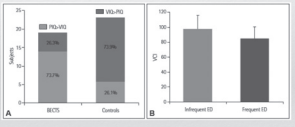

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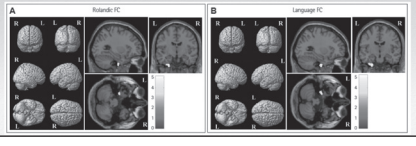
Table 3. Regions exhibiting differences between the patient and control groups in seed-based functional connectivity to voxels throughout the brain

Seed region	ks	Peak level p (FDR-cor)	p (unc)	T score	Coordinate (mm) X	Y	Z	Regions
Rolandic area								
PT>HC	73	0.038*	<0.001	5.159	-28	-10	-44	Left inferior temporal gyrus
HC>PT	98	0.674	<0.001	4.425	-20	12	-10	Left putamen ventroform nucleus
	47	0.674	<0.001	4.211	-20	-76	-26	Left cerebellar posterior lobe, uvula
	75	0.674	<0.001	3.907	50	26	14	Right inferior frontal gyrus
		0.674	<0.001	3.644	48	22	22	Right middle frontal gyrus
	50	0.674	<0.001	3.704	-46	-50	-6	Left temporal fusiform gyrus
		0.908	0.001	3.393	-52	-54	-2	Left inferior temporal gyrus
Language area								
PT>HC	149	0.038*	<0.001	5.281	-28	-12	-46	Left inferior temporal gyrus
	39	0.301	<0.001	4.003	-4	38	-18	Left medial frontal gyrus
HC>PT	128	0.157	<0.001	4.838	50	20	18	Right inferior frontal gyrus

*Statistical significance at FDR-cor p<0.05.
FDR-cor: corrected false discovery rate, HC: healthy controls, ks: cluster, PT: patients, unc: uncorrected.



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Chang-Hyun Park[d,e]
Hye-Sun Hong[a,b]
Yun Sen Choi[a,b]
Jeong Hyun Yoo[f]
Hyang Woon Lee[a,b]

- 1) CECTS patients showed significantly lower cognitive performance as measured by K-WSC-III IQ scores, in addition to different V-P patterns
- 2) Both the Rolandic and Language areas exhibited greater FC to voxels in the left inferior temporal gyrus in CECTS patients than in healthy controls
- 3) The K-WISC-III IQ scores of our subjects were negatively correlated either with Rolandic or language FC to voxel in the left inferior temporal gyrus, demonstrating higher seed based FC values in CECTS patients than in the healthy controls
- 4) The negative tendency of the VCI score combined with the EDs frecuency suggests that poor seizure control has a deleterious impact on cognitive function and maybe the trajectory of neurodevelopmental disruption or secondary pathology induced by the propagation of EDs

Colin J. McGinnity is a PhD student at King's College London in the United Kingdom.

Decreased functional connectivity within a language subnetwork in benign epilepsy with centrotemporal spikes

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Table 2. Demographics and motion indices after exclusions

	BECTS (n = 25) Mean (SD)	Siblings (n = 12) Mean (SD)	Healthy controls (n = 35) Mean (SD)
Age	10.6 (1.6)	11.6 (2.5)	11.9 (1.5)
Male/females	16/9	4/8	12/8
Relative RMS (mm)	0.008 (0.008)	0.006 (0.007)	0.007 (0.007)

BECTS, benign epilepsy with centrotemporal spikes; RMS, root mean square, a measure of movement during the acquisition of the resting functional MRI data; SD, standard deviation.

Table 3. Psychometrics for the groups

	BECTS mean (SD), Cohen's d	Siblings mean (SD), Cohen's d	Healthy controls mean (SD)	(df, error) F, p-value
Intelligence				
WASI Full Scale IQ (n = 24; 12; 30)	109.5 (13.4), 0.4	105.0 (8.0), 1	114.8 (12.5)	(2, 53); 2.6, 0.09
WASI Verbal IQ (n = 24; 12; 30)	109.8 (13.5), 0.1	102.0 (10.7), 0.7	111.3 (14.5)	(2, 53); 2.1, 0.14
WASI Performance IQ(n = 24; 12; 30)	107.0 (14.8), 0.6	107.2 (11.2), 0.7	115.1 (10.5)	(2, 53); 3.4, 0.10
WASI Verbal IQ – Performance IQ absolute difference (n = 23; 12; 30)	12.4 (10.0), 0.2	13.1 (10.9), 0.3	10.1 (8.6)	Kruskal–Wallis p = 0.59
Communication and language				
CCC-2 General Communication Composite (n = 23; 11; 19)	73.3 (14.8), 0.9	80.0 (20.4), 0.7	90.5 (10.8)	(2, 50); 4.3, **0.02***
SCAN A/C Competing Words (n = 24; 11; 18)	90.5 (19.1), 0.3	90.3 (19.1), 0.2	85.7 (19.1)	Kruskal–Wallis p = 0.74
TOWRE-2 Total Word Reading Efficiency (n = 24; 12; 18)	100.1 (13.4), 0.4	97.6 (11.9), 0.7	106.2 (12.4)	(2, 51); 1.9, 0.15
TOWRE-2 Sight Word Efficiency (n = 24; 12; 18)	97.3 (13.8), 0.4	97.3 (12.5), 0.4	103.2 (13.8)	(2, 51); 1.1, 0.33
TOWRE-2 Phonemic Decoding Efficiency (n = 24; 12; 18)	102.7 (13.5), 0.4	98.3 (11.6), 0.9	107.9 (10.6)	(2, 51); 2.4, 0.10
WRAT-4 Reading Composite (n = 25; 12; 20)	108.3 (14.2), 0.4	111.2 (13.7), 0.2	113.8 (10.2)	(2, 54); 1.0, 0.37
WRAT-4 Word Reading (n = 25; 12; 20)	107.8 (13.9), 0.4	111.2 (17.4), 0.1	112.6 (9.8)	(2, 54); 0.7, 0.48
WRAT-4 Sentence Comprehension (n = 25; 12; 20)	108.4 (15.2), 0.4	111.1 (9.7), 0.3	114.2 (13.4)	(2, 54); 1.0, 0.37
WRAT-4 Spelling (n = 24; 12; 20)	112.5 (9.2), 0.2	113.3 (17.6), 0.1	115.4 (14.5)	(2, 53); 0.2, 0.86
Behavioral symptoms				
Conner's Global Impairment (n = 23; 11; 17)	109.2 (20.1), 0.7	99.3 (15.6), 0.2	97.2 (14.9)	Kruskal–Wallis **p = 0.02***
Conner's Attention Deficit Hyperactivity Disorder Index (n = 23; 11; 17)	107.9 (20.5), 0.4	100.7 (13.6), 0.1	99.6 (17.6)	Kruskal–Wallis p = 0.23
SDQ Total Disability* (n = 25; 12; 18)	10.1 (4.8), 0.9	5.8 (3.7), 0.0	5.6 (5.2)	Kruskal–Wallis **p = 0.002****
SDQ Emotional Symptoms (n = 25; 12; 18)	2.4 (1.9), 1.1	0.8 (0.9), 0.1	0.7 (0.7)	Kruskal–Wallis **p = 0.002****
SDQ Conduct Problems (n = 25; 12; 18)	1.5 (1.5), 0.2	1.0 (1.1), 0.2	1.3 (2.0)	Kruskal–Wallis p = 0.51
SDQ Hyperactivity (n = 25; 12; 18)	4.5 (2.9), 0.8	2.8 (2.7), 0.1	3.5 (2.2)	(2, 52); 3.3, 0.04
SDQ Peer Problems (n = 25; 12; 18)	1.7 (1.8), 0.3	1.3 (2.1), 0.1	1.2 (1.4)	Kruskal–Wallis p = 0.36
Coordination				
DCD-Q-07 Total* (n = 24; 10; 19)	56.9 (14.5), 1.0	66.2 (9.3), 0.4	68.9 (7.2)	Kruskal–Wallis **p = 0.005****

Decreased functional connectivity within a language subnetwork in benign epilepsy with centrotemporal spikes

*Colin J. McGinnity, *Anna B. Smith, *Siti N. Yaakub, *Sofia Weidenbach Gerbase, †Anya Gammerman, §§Adam L. Tyson, *Tiffany K. Bell, †Marwa Elmasri, ¶Gareth J. Barker, ** Mark P. Richardson, and ** Deb K. Pal

Epilepsia Open, 2(2):214–225, 2017
doi: 10.1002/epi4.12051

- Relative to controls, patients with CE[illegible]ificant decrease in connectivity ($p<0.05$) with[illegible] superior frontal gyrus – orbital part, the left inferior frontal gyrus – opercular part, the left supramarginal gyrus, and the right inferior parietal lobe
- A significant increase in connectivity was identified in the left and right superior medial frontal regions, the left and right olfactory regions, and the left anterior cingulate gyrus

Broca's area

phonological processing and visual word recongnition

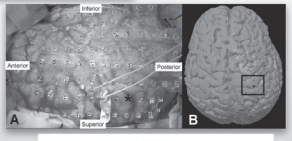
Disruption of Rolandic Gamma-Band Functional Connectivity by Seizures is Associated with Motor Impairments in Children with Epilepsy

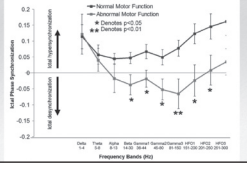
PLoS ONE

George M. Ibrahim[1,2], Tomoyuki Akiyama[2], Ayako Ochi[2], Hiroshi Otsubo[2], Mary Lou Smith[2,4], Margot J. Taylor[2,3,5,6], Elizabeth Donner[7], James T. Rutka[1], O. Carter Snead, III[2,4], Sam M. Doesburg[2,5,6],*

June 2012 | Volume 7 | Issue 6 | e39326

- Seizures alter the functional connectivity of eloquent cortical areas and these alterations are predictive of clinical neurological deficit
- Ictal phase desynchronization and disruption of functional connectivity within a variety of distributed brain networks may underlie the broad spectrum of impairments affecting children with epilepsy
- The most significant frecuency was the high gamma band (81-150 Hz) ($p<0.01$), suggesting that ictal desynchtonization was strongest within the gamma band, which has been reliably implicated in the formation of networks supporting cognition, perception and motor control
- Role of interictal discharges in disrupting networks beyond the ictal period, further contributing to network destabilization

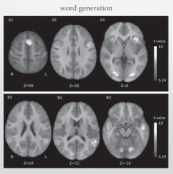


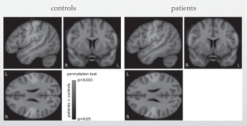


Reduced functional integration of the sensorimotor and language network in rolandic epilepsy[17]

René M.H. Besseling [a,b,c,1], Jacobus F.A. Jansen [b,c], Geke M. Overvliet [a,b,1], Sylvie J.M. van der Kruijs [a,b,1], Johannes S.H. Vies [a,d,1], Saskia C.M. Ebus [a,1], Paul A.M. Hofman [a,c,1], Anton de Louw [a,1], Albert P. Aldenkamp [a,d,1], Walter H. Backes [b,c,*]

NeuroImage: Clinical 2 (2013) 239–246





- Authors aim to link epileptiform activity/seizures originating from the rolandic cortex with language impairment in children with RE using fMRI, in resting state and during task (word generation and reading)
- Rolandic network covered bilateral pre and postcentral gyri, but in addition included perisylvian regions, as well as bilateral cerebellar and medial regions, and left prefrontal region, which was absent at the right
- This region corresponded to the left inferior frontal gyrus showing activation for the word generation task, and had significantly reduced rolandic network connectivity in patients compared to controls
- This functional decoupling might be key in understanding RE typical language impairment, and is in line with the identified neuropsychological profile of anterior language dysfunction

Epilepsy Behav. 2015 December ;53:174–179. doi:10.1016/j.yebeh.2015.10.016.

Risk factors for reading disability in rolandic epilepsy families

Yazia Hernández Vega[1], Anna Smith[1], Hannah Cockerell[2], Shan Tang[1,2], Zahra Agirre-Arrizubieta[3], Nashmia Goyal[2], Marina Price[2], Ozgünen i Akman[2], Nicola Julian[2], Colin McClimenty[2], Kurnudai Gomez[2], Rajesh Gupta[2], Elaine Hughes[2], John Jackman[2], David McCormick[2], Caroline Owen[2], David Scott[2], Jacqueline Taylor[2], John Trounce[2], Tara Clarke[2], Steven Kugler[2], David E Mandelbaum[2], Patricia McGoldrick[2,4], Steven Wolf[4], Lisa J Shugg[2,5], and Deb K Pal[1,2]

- RD in RE is strongly associated with history of speech sound disorder (SSD), ADHD, and male sex among probands
- RD in RE is very common (43% probands vs 22% siblings, and often preceded by SSD (53% probands, 28% siblings)
- When ADHD is reported in probands, it is usually associated with RD (84%)
- Within the context of RE, neither seizure number or early seizure onset, nor treatment factors, increase risk for RD

Rolandic epilepsy and dyslexia

Epilepsia rolândica e dislexia

Ecila P. Oliveira, Marina L. Neri, Livia L. Capelatto, Catarina A. Guimarães, Marilisa M. Guerreiro

Table 1. Comparison of dyslexia diagnosis in the group of patients with benign epilepsy with centrotemporal spikes (BECTS) and the control group.

Group	DH		
	Dyslexia*	OD	WD
A	6 (19.4%)	23 (74.20%)	2 (6.50%)
B	0 (0.0%)	11 (35.5%)	20 (64.50%)
Total	6 (9.70%)	34 (54.90%)	22 (35.50%)

*p<0.001. A: Patients with BECTS; B: Control group; DH: Diagnostic hypothesis; OD: Other difficulties, WD: Without difficulties. Chi-square test.

- Patients diagnose with dylexia had a lower performance in reading and writing and average perfomance in arithmetic, a condition consistent with the diagnosis of dylexia
- The six patients diagnosed with dyslexia, the worst scores found were related to verbal memory and learning, while visual memory remained within average or above average

Increased resting-state EEG functional connectivity in benign childhood epilepsy with centro-temporal spikes

Béla Clemens[a], Szilvia Puskás[b,*], Tamás Spisák[c], Imre Lajtos[c], Gábor Opposits[c], Mónika Besenyei[a], Katalin Hollódy[a], András Fogarasi[a], Noémi Zsuzsanna Kovács[a], István Fekete[d], Miklós Emri[c]

Seizure 35 (2016) 50–55

- Abnormal EEGfC in frontal areas: Abnormally increased neuronal coupling between frontal and frontal, frontal and temporal regions. We found increased bilateral beta band connectivity. Decreased hemodynamic coupling together with increased electrical coupling (EEGfC) is common finding in focal epilepsy. These abnormalities are presumably pathophysiologically related to specific **language deficit**, the neuropsychological endophenotype of RE
- Abnormal EEGfC in the parietal area: Increased EEGfC within the right parietal area. This abnormality was topographically limited but involved the entire investigated frecuency spectrum, so it should be considered as neurophysiologically important. It topographically corresponds to the supeior parietal area, an important node of the **attention network**. **Attention deficit** due to superior parietal dysfunction is part of the neuropsychological profile of RE
- No abnormal EEGfC in the central area: It was surprising that EEGfC was normal in the central region that generates spikes and seizures in CECTS. CECTS differs from the rest of focal epilepsies in this respect.

Childhood Epilepsy with Centrotemporal Spikes (CECTS)

- Prevalence of ADHD: healthy school children (3 – 5%); children with epilepsy (8-33%) [1]
- Many studies have suggested various pathogenetic mechanisms of ADHD including a common genetic predisposition or biochemical factor in children with epilepsy [2]
- Bilateral CTSs are significantly more frequent in patients with treated ADHD
  - Perhaps, left lateralized language and auditory processing, and typically right lateralized sustained attention are both disrupted by bilateral CTSs, and these combined deficts produce more severe ADHD symptoms [3]
- Although the pathophysiology of attention impariment in children with CECTS is still unknown, the overlap between neural circuity for attention and the networks involved in the generation of rolandic seizures may be the basis for this association [4,5]

1- Dunn DW, Austin JK, Haverkamp F, Ambrosius WT. Dev Med Child Neurol 2003;45:50-4
2- Parisi P, Moavero R, Verrotti A, Curatolo P. Brain Dev 2010;32:10-6
3- Eun-Hee K, M-Sun Y, Hyo-Won K, Tae-Sung K. Epilepsy Behav 2014;37:54-58
4- Paridw HR, Berg AT, Archer JS, et al. Epilepsy Res 2013;105:133-9
5- Besseling RM, Jansen JF, Overvliet GM, et al. PLoS One 2013;8:e83668

Epilepsy & Behavior 53 (2015) 78–82

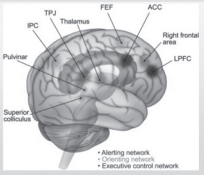
The attentional networks in benign epilepsy with centrotemporal spikes

Bin Yang [a,b], Xiaocui Wang [b], Liwei Shen [b], Xiaofei Ye [b], Guang-e Yang [b], Jin Fan [c], Panpan Hu [a,*], Kai Wang [a,*]

Table 1
The clinical details of children with BECTS (N = 90).

Variables	N (%)/mean ± SD
Gender (female)	33 (36.7)
Duration of clinical course (months)	4.55 ± 8.72
The ANT test ages (years)	8.47 ± 1.72
Age at first seizure (years)	7.08 ± 1.81
Family history of convulsions (yes)	12 (13.3)
Febrile seizures (yes)	4 (4.4)
Consciousness (existence)[c]	16 (17.8)
Oropharyngeal symptoms (yes)[c]	65 (72.2)
Ending with hemi or generalized (yes)[c]	71 (78.9)
Total number of seizures	
1	34 (37.8)
2	24 (26.7)
≥ 3	32 (35.5)
Frequency of seizure[a] (seizures per month)	
Mild (≤1)	35 (38.9)
Moderate (1–3)	25 (27.8)
Severe (≥3)	30 (33.3)
Duration of seizure[b]	
≤1 min	13 (14.4)
1–10 min	73 (81.8)
≥10 min	4 (4.4)
EEG	
Normal	3 (3.3)
Left hemisphere spikes	32 (36.6)
Right hemisphere spikes	34 (37.8)
Bilateral hemisphere spikes	21 (23.3)
Spike index	
Awake stage	5.20 ± 5.45
NREM sleep stage	28.88 ± 19.25
Brain image data	
Normal CT findings	23 (25.6)
Normal MRI findings	67 (74.4)

Note. ANT: attention network test.
[a] Frequency of seizures is defined as the average number of seizures per month.
[b] Duration of seizures is an estimation reported by parents of patients, according to their last seizure.
[c] Refer to ictal symptoms in BECTS.



- The attentional system can be divided into three subsystems: alerting, orienting, and executive control, based on their neuroanatomical and physiological mechanisms, amid these three subsytems are coordinated to complete the entire information procession of attentional networks

The attentional networks in benign epilepsy with centrotemporal spikes

Bin Yang [a,b], Xiaocui Wang [b], Liwei Shen [b], Xiaofei Ye [b], Guang-e Yang [b], Jin Fan [c], Panpan Hu [a,*], Kai Wang [a,*]

Epilepsy & Behavior 53 (2015) 78–82

Table 2
Comparisons of ANT performance between children with BECTS and controls.

Variables	BECTS (N = 90)	Controls (N = 90)	U	Z	P
Alerting effect (ms)	35.30 ± 13.75	42.58 ± 23.45	3160.50	−2.545	0.011
Orienting effect (ms)	24.19 ± 15.55	47.80 ± 27.36	1916.50	−1.605	**<0.001**
Conflict effect (ms)	76.20 ± 14.34	72.76 ± 14.18	3489.00	−1.605	0.108
Grand mean effect (ms)	936.23 ± 147.82	713.53 ± 108.55	959.50	−8.842	**<0.001**
Accuracy (%)	95.71 ± 5.85	98.57 ± 1.73	2294.00	−5.119	**<0.001**

Data are presented as mean ± SD. P-value represents the results of Mann–Whitney U-test.

- This study found that RE mainly affected the orienting function, the grand mean effect, and the accuracy
- Various degrees of impairments related to the orienting function, such as the visuomotor coordination and the visuospatial capacities, have been reported in children with RE

The attentional networks in benign epilepsy with centrotemporal spikes

Bin Yang [a,b], Xiaocui Wang [b], Liwei Shen [b], Xiaofei Ye [b], Guang-e Yang [b], Jin Fan [c], Panpan Hu [a,*], Kai Wang [a,*]

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Table 3
Comparisons of ANT performance in children with BECTS under and above 8 years old.

Variables	BECTS		U	Z	P
	≤8 ages (N = 50)	>8 ages (N = 40)			
Alerting effect (ms)	34.66 ± 13.38	36.10 ± 14.34	938.00	−0.504	0.615
Orienting effect (ms)	20.38 ± 14.26	28.95 ± 19.95	697.00	−2.461	**0.014**
Conflict effect (ms)	76.64 ± 13.55	75.65 ± 15.64	974.00	−0.211	0.833
Grand mean effect (ms)	998.24 ± 133.00	863.72 ± 133.82	485.50	−4.176	**<0.001**
Accuracy (%)	94.86 ± 6.44	96.78 ± 4.88	681.00	−2.630	**<0.01**

Data are presented as mean ± SD. P-value represents the results of Mann–Whitney U-test.

Table 4
Multiple linear regression of accuracy and the grand mean effect.

	Accuracy			The grand mean effect		
	β	SE	P	β	SE	P
The age of onset	1.49	0.33	<0.001	−34.23	8.48	<0.001
Awake spike index	0.11	0.11	0.325	1.48	2.80	0.599
Sleep (NREM stage) spike index	−0.07	0.03	0.016	−1.44	0.78	0.067

Adjusted: gender, total number of seizures, duration of seizures.

- The age at onset of seizures was highly correlated with the accuracy and the grand mean effect. The younger the children with RE were, the lower the accuracy and the longer the grand mean effect
- Centrotemporal spikes were associated with widespread adverse effects on attentional networks

♦ Human Brain Mapping 00:00–00 (2016) ♦

Interictal Activity is an Important Contributor to Abnormal Intrinsic Network Connectivity in Paediatric Focal Epilepsy

Elhum A. Shamshiri,[1,*] Tim M. Tierney,[1] Maria Centeno,[1] Kelly St Pier,[2] Ronit M. Pressler,[2,4] David J Sharp,[1] Suejen Perani,[1,4] J Helen Cross,[2,4] and David W. Carmichael[1]

- The goal of treatment in epilepsy is seizure freedom. However, the benefits of IEDs suppression are controversial as the evidence for the impact of IEDs on cognitive function is mixed
- IED prevalence is not typically used as an indication for treatment modification
- The increased rate of epileptiform discharges has been associated with lower performance on cognitive functioning and attention-sensitive tasks which is dependent on when and where the activity occurs
- Non transient effects of IEDs are less well characterised although there is some evidence that a worse cognitive outcome in the long term is related to increased frecuency of epileptic discharges in focal epilepsies

• Human Brain Mapping 00:00–00 (2016) •

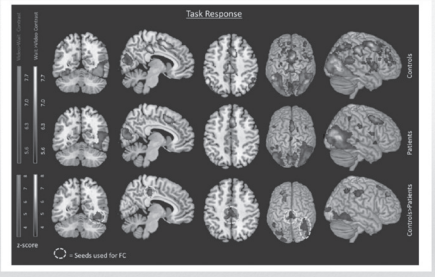
Interictal Activity is an Important Contributor to Abnormal Intrinsic Network Connectivity in Paediatric Focal Epilepsy

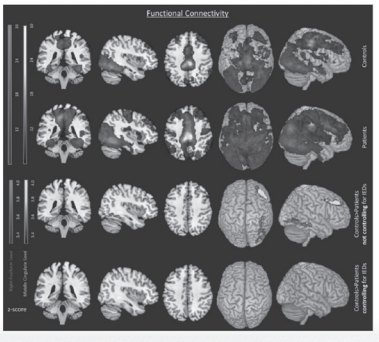
Elhum A. Shamshiri,[1,6] Tim M. Tierney,[1] Maria Centeno,[1] Kelly St Pier,[2] Ronit M. Pressler,[1,4] David J Sharp,[3] Suejen Perani,[1,4] J Helen Cross,[1,5] and David W. Carmichael[1]

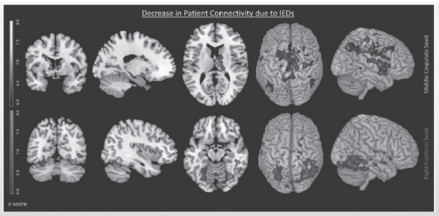
Patient	Gender	Age	Total IEDs	Age of onset (years)	Epilepsy focus localisation (for ictal onset)	Lesion type	IQ (FSIQ)	Medication (mg/day)
# 1	Female	14	0	4	Left Frontal	None	81	CBZ 800, LVT 2000
# 2	Male	11	255	0.25	Left Temporal	Hypothalamic Hamartoma	88	LVT 1625
# 3	Male	15	0	10	Left Temporal	None	108	CBZ 600
# 4	Female	11	66	1.3	Right Fronto-Temporal Junction	None	93	LCM 350 GAB 500 CLBZ 5
# 5	Male	15	44	10	Right Frontal	None	83	CBZ 1200
# 6	Male	14	478	8	Left Temporal Posterior	Focal Cortical Dysplasia	84	OXC 1200, LVT 1000
# 7	Female	14	181	2.5	Right Temporal	Cortical Abnormality Unknown Aetiology	N/A	LVT 2000, TPM 150
# 8	Female	11	265	6	Right Fronto-temporal	None	66	CBZ 800, LTG 250
# 9	Female	10	270	7	Right Fronto-Polar	Focal Cortical Dysplasia	115	OXC 1050
# 10	Female	16	168	10	Right Frontal	None	111	LTG 500, LVT 2000
# 11	Female	16	21	6	Left Frontal	None	83	VPA 2000, CBZ 400
# 12	Female	17	0	0.42	Left Temporal	Astrocytoma	44	TPM 500, OXC 2100
# 13	Female	15	200	13	Left Insula-deep	Focal Cortical Dysplasia	N/A	TPM 50, CBZ 800
# 14	Male	17	57	0.008	Right Precuneus	Focal Cortical Dysplasia	59*	PGB 250, LCM 400, LTG 300, TPM 200
# 15	Male	11	264	3	Right Frontal	None	56	CBZ 640
# 16	Male	15	234	9	Temporal-posterior quadrant	Hippocampal Sclerosis	91	LTG 575, ZNS 200
# 17	Female	17	134	3	Left Temporal	None	56	LVT 3000
# 18	Female	16	0	5	Left Frontal	None	109	OXC 1200
# 19	Male	15	160	8	Right Frontal	None	119	PMP 4
# 20	Male	12	44	3	Right Frontal	Focal Cortical Dysplasia	105	OXC 1050, CLBZ 6
# 21	Female	16	88	5	Right Frontal	None	80	OXC 1250, CBZ 280
# 22	Male	10	104	6	Left Frontal	None	87	OXC 1250
# 23	Male	16	333	8	Right Parietal	None	87	LVT 2000, OXC 1800, CLBZ 20
# 24	Female	17	541	5	Bilateral Orbito-Frontal	Bilateral orbitofrontal cortex polymicrogyria	101	LVT 2000, VPA 800
# 25	Female	17	0	1	Right Fronto-Parietal	Focal Cortical Dysplasia	57	OXC 2100, TPM 200, RUF 2400
# 26	Female	11	692	5	Right Parietal	Focal Cortical Dysplasia	81	OXC 1500, CLBZ 10, VPA 1000
# 27	Female	16	146	12	Left Frontal Midline	None	102	VPA 1000

- The aim of the study was to provide a detailed investigation on the impact of IEDs in paediatric focal epilepsy by measurements of network connectivity, known to be a possible marker of cognitive performance
- Two hypothesis:1) epilepsy patients would have reduced functional connectivity within networks angaged by the natural stimulus task; 2) functional connectivity would increase in epilepsy patients after the removal of fMRI signal changes related to IEDs

Figure 1.
Task paradigm.







- This suggests that there is a common pathway through which IEDs can impact cognitive networks and subsequently performance across focal epilepsy patients with different localizations
- The results presented here demonstrate that there are significant neurobiological changes known to predict brain function that were associated with IEDs even during a low demand cognitive task
- The results also raise the question that if IEDs were supressed by treatment in the paediatric setting, would an improvement in cognition be possible via the restoration of cognitive network connectivity?

Epileptic Discharge Related Functional Connectivity Within and Between Networks in Benign Epilepsy with Centrotemporal Spikes

Rong Li*, Gong-Jun Ji†, Yangyang Yu*, Yang Yu‡, Mei-Ping Ding§, Ye-Lei Tang§, Huafu Chen* and Wei Liao*,¶

International Journal of Neural Systems, Vol. 27, No. 0 (2017)

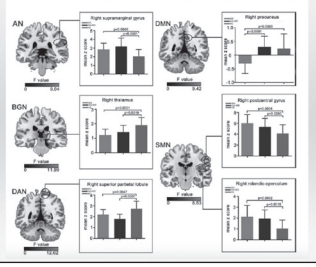
- The aim of this study was to examine alterations in functional connectivity both within and between networks in RE patients with IEDs and without IEDs. We hypothesized altered functional connectivity both within and between ICNs corresponding to seizure origination and cognitive processes in patients with RE

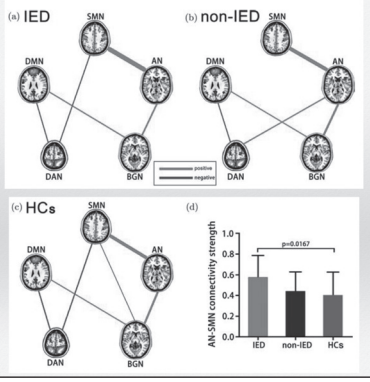
Table 1. Demographic and clinical information of subjects.

	IED ($n = 20$)	non-IED ($n = 23$)	HC ($n = 28$)	p value
Age (years)	9.00 ± 1.95	10.22 ± 2.13	10.00 ± 2.31	0.63^a
Sex (female: male)	13:7	11:12	13:15	0.40^b
Onset age (years)	6.95 ± 1.85	7.48 ± 2.43	—	0.43^c
Duration (months)	23.28 ± 31.90	34.27 ± 35.92	—	0.32^d
Side of EEG (L:R:Bil)	6:13:1	10:11:2	—	0.52^e
Number of seizures (/year)	2.27 ± 2.13	6.12 ± 11.75	—	0.42^d
Treatment : Naïve	10:10	17:6	—	0.11^e
Medication (LEV:VAL:LTG:OXC)	2:2:2:5	6:4:4:3	—	0.45^e
IQ				
Full-scale IQs	111.80 ± 11.52	107.40 ± 14.85	113.80 ± 14.59	0.31^a
Verbal IQ	107.50 ± 14.05	105.00 ± 16.54	114.50 ± 15.84	0.13^a
Performance IQ	113.90 ± 13.38	108.70 ± 16.51	109.50 ± 14.36	0.52^a
mean FD (mm)	0.08 ± 0.06	0.06 ± 0.03	0.09 ± 0.06	0.15^a

Table 2. Brain regions showing significant group differences of functional connectivity within each ICN.

Brain regions	MNI coordinates (x, y, z)	Voxels	ANOVA F-value	IED versus HC p-value	non-IED versus HC p-value	IED versus non-IED p-value
AN (cluster> 19)						
R Supramarginal gyrus	(39, −33, 24)	31	9.04	0.0049(↑)	< 0.0001(↑)	—
BGN (cluster> 14)						
R Thalamus	(9, −27, 3)	36	11.99	< 0.0001(↓)	0.0019(↓)	—
DAN (cluster> 24)						
R Superior parietal lobule	(15, −48, 69)	86	12.62	0.0047(↓)	< 0.0001(↓)	—
DMN (cluster> 22)						
R Precuneus	(9, −51, 33)	31	9.42	0.0009(↓)	—	< 0.0001(↓)
SMN (cluster> 20)						
R Postcentralgyrus	(51, −12, 36)	45	8.27	0.0004(↑)	0.0299(↑)	—
R Rolandic operculum	(51, −15, 21)	39	8.53	0.0002(↑)	0.0019(↑)	—





Epileptic Discharge Related Functional Connectivity Within and Between Networks in Benign Epilepsy with Centrotemporal Spikes

Rong Li[a], Gong-Jun Ji[1], Yangyang Yu[a], Yang Yu[1], Mei-Ping Ding[1], Ye-Lei Tang[1], Huafu Chen[a] and Wei Liao[a,¶]

- The results support the proposed network inhibition hypothesis, which suggests that epileptiform activity arising from the epileptic focus (and subsequently propagating to subcortical stuctures such as medial thalamus and upper brainstem) may lead to inhibition of nonseizing cortical cortices
- The current study found that RE patients with IEDs exhibited increased positive connectivity between the AN and the SMN compared with the non IED group and HCs
- The sensorimotor cortex is thought to be the neural source of centrotemporal epileptiform activity, and sensorimotor cortex to one or more functionally interconnected areas
- The downstream effects of epileptic activity may this eventually lead to disruption of functional neural systems and normal function of other brain areas in children with RE

Conclusion

- Neurocognitive impairment in CECTS is common and related to abnormal functional connectivity that involves regions remote from sensorimotor cortices, which suggests that CECTS is a network disorder, genetically influenced

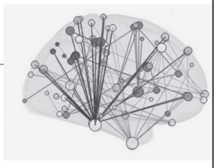
Marina Bentivoglio (Italy)

SCIENCE IN THE 21ST CENTURY: ACHIEVEMENTS, PROBLEMS, NEED FOR INTEGRITY

Alicia Bogacz (Uruguay)

IS SEIZURE CLASSIFICATION COMPATIBLE WITH THE CONNECTOME CONCEPT?

IS SEIZURE CLASSIFICATION COMPATIBLE WITH THE CONNECTOME CONCEPT?



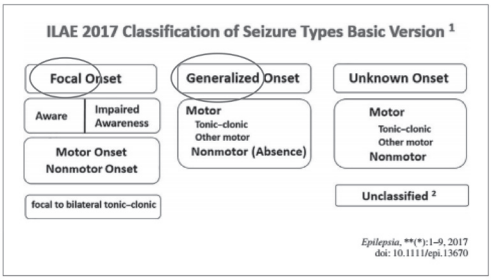
Dra. Alicia Bogacz
Sección Epilepsia Instituto de Neurología
Universidad de la República
Montevideo-Uruguay
LASSE 2019

What it is a seizure?

Epilepsy is a sudden excessive and rapid discharge of grey matter of some part of the brain, it is a local discharge.
(John Hughlinds Jackson, 1873)

- An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain.
(Fisher, R. et al., 2005)

- What are the spatial and temporal boundaries of seizure activity in brain networks?
- Network interactions give rise to abnormal activity in local circuits, more or less extend, and can involved remote brain regions.
- Are these remote network changes part of the seizure or are they “side effects” caused by the seizure but not directly involved in the seizure network?



- **"Focal epileptic seizures** are originating within networks limited to one hemisphere. They may be localized or more widely distributed. Focal seizures may originate in subcortical structures."
- For each seizure type, ictal onset is consistent from one seizure to another, with preferential propagation patterns that can involve the contralateral hemisphere."
- **"Generalized epileptic seizures** are originating at some point within, and rapidly engaging, bilaterally distributed networks. Such bilateral networks can include cortical and subcortical structures, but do not necessarily include the entire cortex."

(Berg A et al. Epilepsia, 51(4):676–685, 2010)

- The terms ''focal'' and ''generalized'' express a dichotomous based on current electro-clinical evidence.
- A distinction between focal and generalized onset is a practical one , and may change with advances in ability to characterize the onset of seizures.

(Fisher RS et al. Epilepsia, 58(4):522–530, 2017)

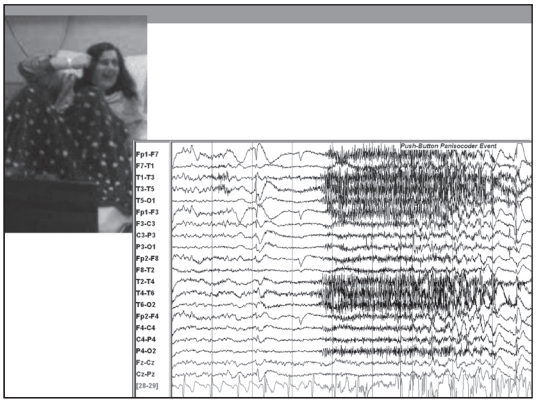
FOCAL SEIZURES

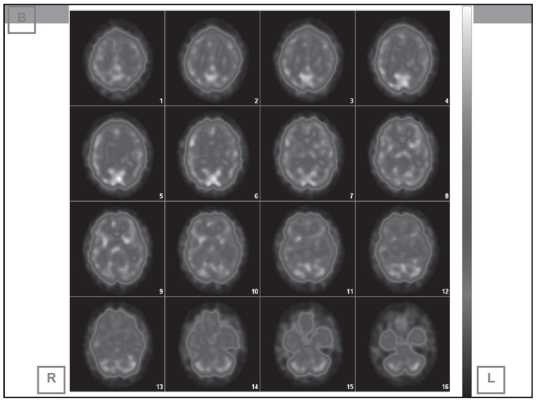
- "Focus" concept was developed with the records using scalp-EEG or subdural grids.
- With SEEG the concept of epileptogenic zone was developed as interrelated brain zones that are involved in the primary organization of the ictal discharge.
- Seizures are the expression of a sufficient number of neurons connected in a network.

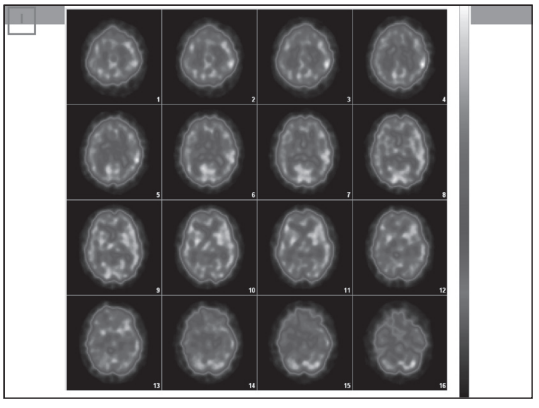
What is the evidence?

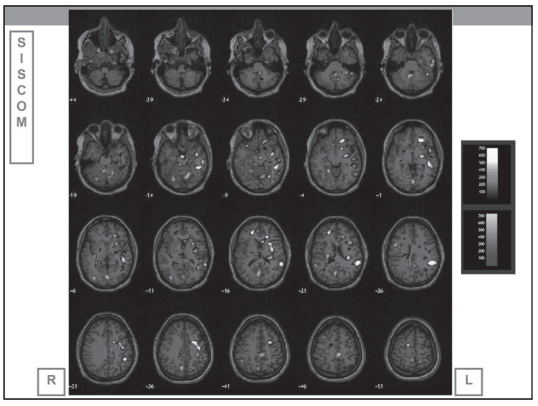
ICTAL

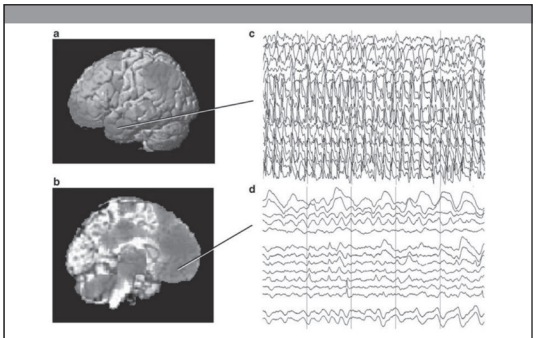
- Scalp-EEG or subdural grids
- Stereo-EEG
- Ictal-SPECT





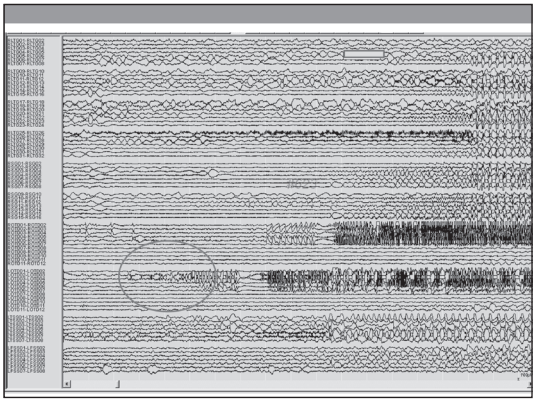


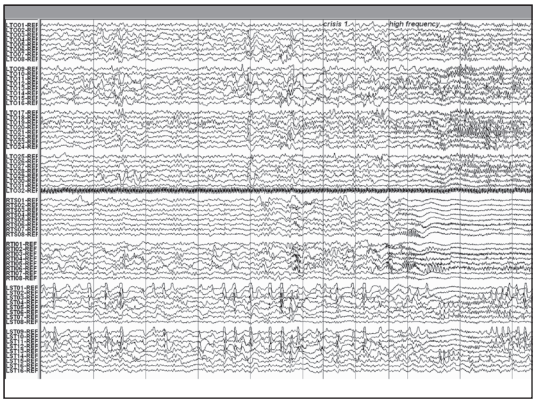


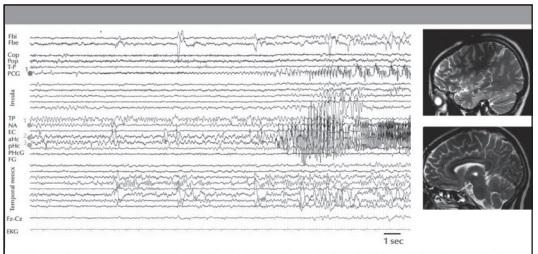


Group analysis of SPECT ictal-interictal difference imaging during temporal lobe seizure

(Blumenfeld H, Adv Exp Med Biol. 2014)



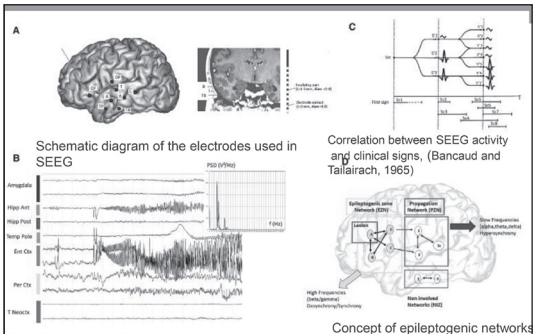




During the seizure, SEEG revealed a low-voltage fast activity involving the parietal cingulate gyrus, which preceded the mesio-temporal discharge.

Epileptic activity starts on the PCG (posterior cingulate gyrus) contact and propagates to mesial temporal structures (NA: amygdala; aHc: anterior hippocampus ; pHc: posterior hippocampus).

Theys T. et al. Epileptic Disord 2018; 20 (1): 1-12



Schematic diagram of the electrodes used in SEEG

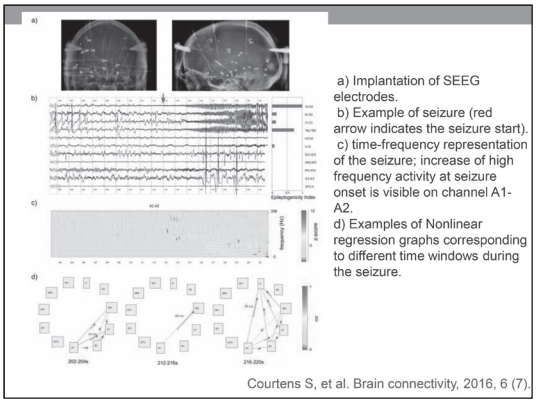
Correlation between SEEG activity and clinical signs, (Bancaud and Talairach, 1965)

Concept of epileptogenic networks

SEEG recordings of ictal discharge in amygdala, anterior hippocampus, posterior hippocampus and entorhinal cortex

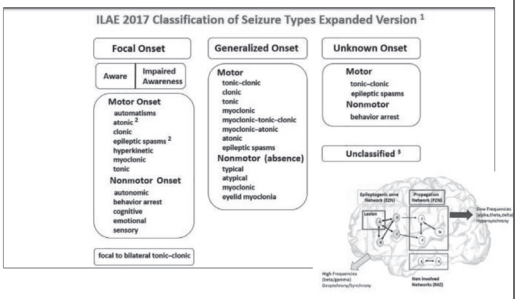
Bartolomei F. et al. Epilepsia, 58(7):1131–1147, 2017

- The first clinical signs are observed after electrical seizure onset and are often related to propagation of the discharge.
- Ictal clinical symptoms could be related to the abnormal activation of physiologic neural networks or to the disruption of mechanisms governing normal brain function.
- Assessing the extent of the epileptogenic zone and its organization could requires advanced methods of signal processing.



a) Implantation of SEEG electrodes.
b) Example of seizure (red arrow indicates the seizure start).
c) time-frequency representation of the seizure; increase of high frequency activity at seizure onset is visible on channel A1-A2.
d) Examples of Nonlinear regression graphs corresponding to different time windows during the seizure.

Courtens S, et al. Brain connectivity, 2016, 6 (7).



Need descriptors to define the network.

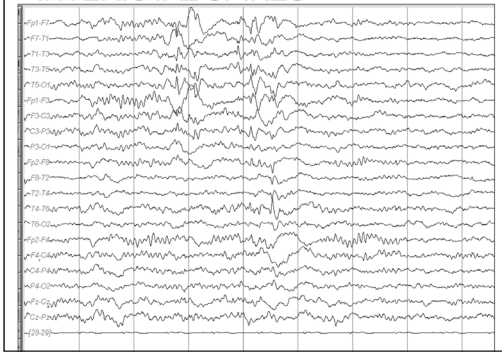
INTERICTAL

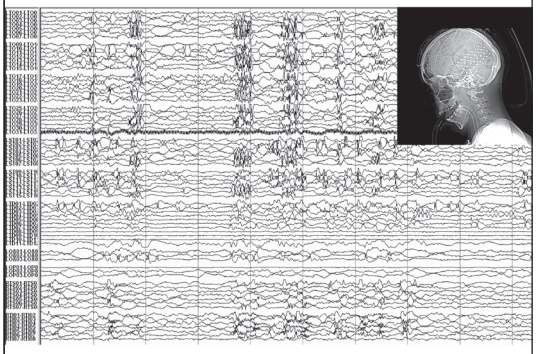
- EEG
- FDG-PET
- EEG-fMRI
- f-MRI : Resting state networks

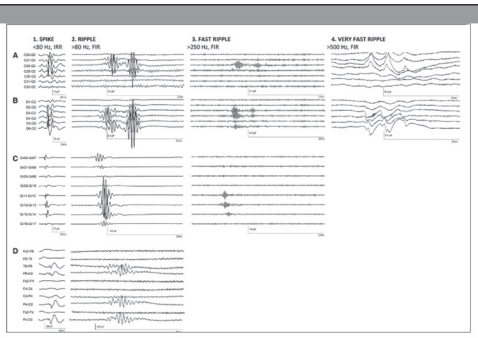
➢During the interictal state there are electro-physiologic biomarkers of the pathologic process:

- **Epileptic spikes** closely related to the epileptogenic zone ("irritative zone") but can appear in regions remote from the epileptogenic zone (propagation networks).
- **High-frequency oscillations** (HFOs) can be a biomarker for the delineation of the seizure-onset zone.
(Bragin et al. 2010;Zijlmans et al. 2012)

INTERICTAL SPIKES

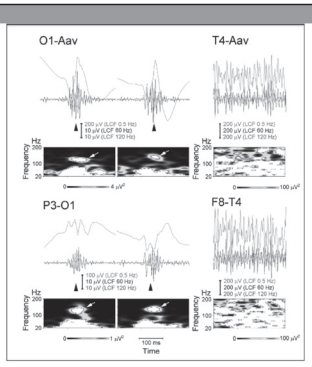






HFOs recorded with different methods: A) Long-term ECoG. B) Long-term depth recording C) Intraoperative ECoG. D) Scalp-EEG .

Zijlmans M. Et al. Epilepsia, 58(8):1305–1315, 2017

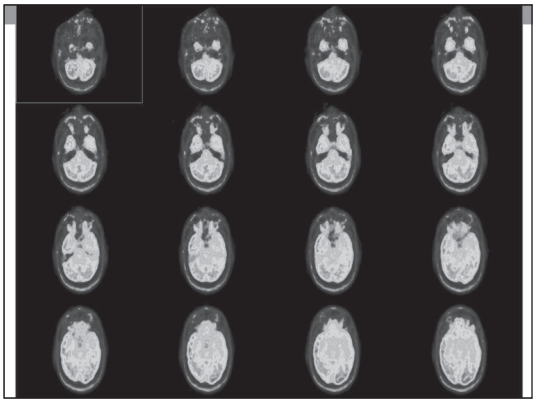


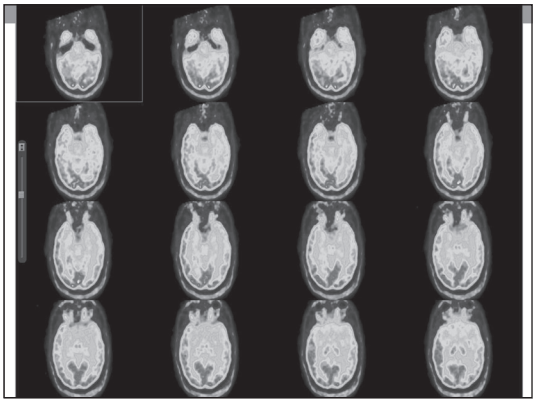
Ripple oscillations in scalp-EEG from a child with Landau-Kleffner syndrome.

Worrell GA et al. Prog Neurobiol.2012 Set; 98(3): 265-278.

FDG-PET

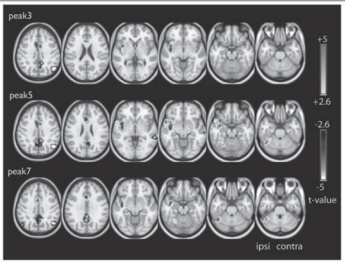
- It shows areas of hypo-metabolism that are the expression of dysfunction usually more extended than the lesion visible in the MRI.





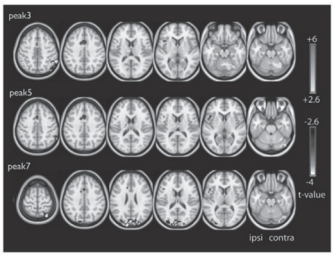
EEG-fMRI

- EEG-fMRI could explore the whole brain noninvasively at the time of a discharge.
- Focal IEDs recorded from scalp EEG may represent only a fraction of events that involve widespread brain areas despite their focal appearance.
- Studies revealed widespread activations and deactivations outside the epileptic focus.



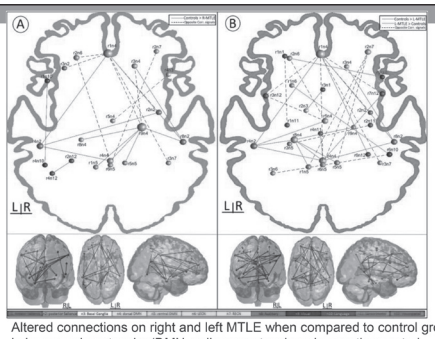
Temporal lobe interictal discharges showing: Activation clusters in the mid–cingulate gyri bilaterally, and in the ipsilateral insula, mesial and lateral temporal regions, and cerebellum. Significant deactivations are found bilaterally in the inferior parietal lobules, posterior cingulate cortex, and precuneus and in the contralateral posterior temporal cortex.

Fahoumet F. et al. Epilepsia, 53(9):1618–1627, 2012



Frontal lobe interictal discharges showing: Activation clusters bilaterally in the cingulate gyri, and in the ipsilateral frontal operculum, medial thalamus, and internal capsule as well as the contralateral cerebellum. Significant deactivation are found in the contralateral inferior and superior parietal lobules and in the cunei bilaterally.

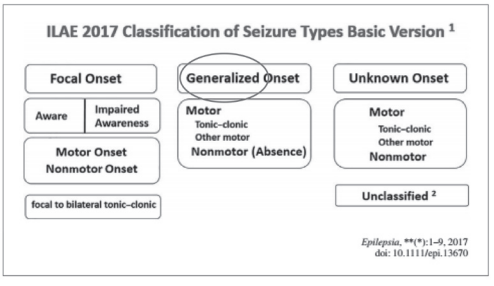
Fahoumet F. et al. Epilepsia, 53(9):1618–1627, 2012



Altered connections on right and left MTLE when compared to control group, in large-scale networks (DMN, salience network and executive control network)
(A) Altered connections on R-MTLE. (**B**) Altered connections on L-MTLE.
(de Campos BM et al. Hum Brain Mapp. 2016 Sep;37(9):3137-52.)

- An epileptic brain is diffusely abnormal, even in the context of focal seizures, and that distance abnormalities and their connections contribute to seizure generation and associated cognitive and behavioral morbidity.

(Scott R , et al. Epilepsia 2017)



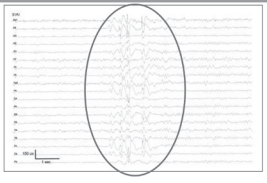
GENERALIZED SEIZURES

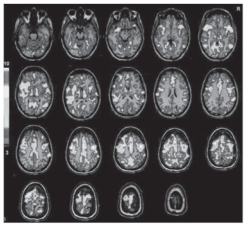
- Generalized seizure are events for which no side can be identified from the onset.
- They involve both cortical and subcortical circuits, in both hemispheres from the onset.
- In many instances are defined by EEG .
- Can be either symmetrical or asymmetrical.
- They consist of absences or motor seizures: tonic, atonic, clonic, tonic–clonic, myoclonic seizures; or spasms.
- Each of these seizure types involves a distinct hyper-excitable circuitry that in most instances does not comprise the whole brain.

SYSTEM EPILEPSIES

- Epilepsies where the ‘‘enduring propensity to generate seizures’’ is due to the **specific susceptibility of a system,** although it may be possible to identify some **trigger areas** within the system.
- EEG-fMRI studies have shown the involvement of neural networks in spike and wave discharges in generalized epilepsy, supporting the idea of a hyperexcitable cortico-subcortical network, consisting of well-defined brain regions, rather than the expression of generalized brain dysfunction.

Avanzini G. et al. Epilepsia,53(5):771–778,2012

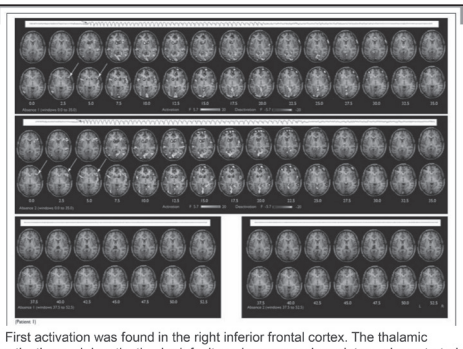




Childhood absence epilepsy:

- GSW burst recorded inside the MRI scanner.
- fMRI results superimposed on anatomical MRI showing diffuse cortical fMRI activation, as well as thalamic activation.
- The cortical activation involves widespread cortical areas(superior and mesial frontal regions).

Aghakhani Y *Brain*, Volume 127 (5,) 2004: 1127–1144

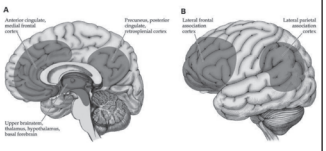


First activation was found in the right inferior frontal cortex. The thalamic activation and deactivation in default mode areas and caudate nucleus started between 5 s and 7.5 s after onset. Thalamic activation was followed by thalamic deactivation.

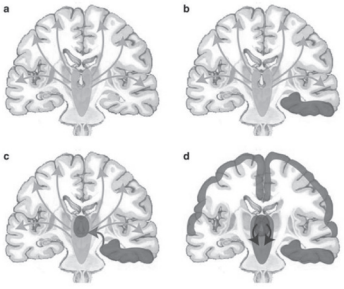
Moeller F. et al Epilepsia,51(10):2000–2010,2010

Aware and impairment of awareness

- Awareness knowledge of self and environment.
- Normal cortical function and awareness is maintained by interactions between the cortex and subcortical arousal systems including the thalamus, brainstem and basal forebrain.
- Seizures disrupt this physiologic interaction through abnormal increases, decreases, or altered patterns of neural activity.

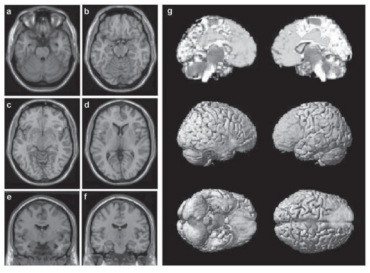


Blumenfeld H Lancet Neurol. 2012 Sep; 11(9): 814-826



a) Normal cortical function and consciousness is maintained by interactions between the cortex and subcortical arousal systems.
b) Focal temporal lobe seizure activity in simple partial seizures does not have long-range network impact effects.
c) In temporal lobe complex partial seizures, propagation to subcortical structures.
d) Inhibits subcortical arousal systems (anterior hypothalamus, lateral septum and other regions).

Englot D. et al. Journal of Neuroscience, 2009, 29 (41) 13006-13018



- **A-D.** Horizontal sections progressing from inferior to superior.
- **E, F.** coronal sections progressing from anterior to posterior showing **blood flow increases in the bilateral midbrain, hypothalamus, medial thalamus, and midbrain**.
- **Decreases are seen in the bilateral association cortex**.

Complex partial seizures arising from the temporal lobe are associated with significant cerebral blood flow increases and decreases in widespread brain regions.

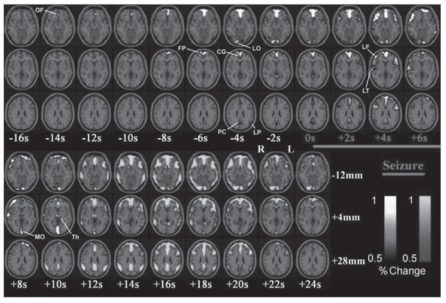
Blumenfeld H et al. Cereb Cortex 2004 14(8):892–902

- SPECT imaging suggest a mechanistic link between subcortical changes and depressed cortical function in temporal lobe seizures.
- Long-range network changes in cortical and subcortical function are seen specifically in temporal lobe seizures with impaired awareness.
- Temporal lobe seizures without impaired awareness are associated with localized seizure activity in the temporal lobe, without these long-range network changes.

Blumenfeld H et al. Cereb Cortex 2004 14(8):892–902

- Simultaneous EEG-fMRI studies in absence seizures have found:

- -increases in the thalamus.
- -decreases in the medial frontal, medial parietal, anterior/posterior cingulate, and lateral parietal cortex.
- -mixture of increases and decreases in the lateral frontal cortex.



Absence seizures: early and late fMRI changes in cortical-subcortical networks (Bai X et al, 2010, Journal of Neuroscience 30:5884-5893.)

CONCLUSIONS

- The dichotomies of the seizure classification are no sufficient to included all the new information, for this raison it is include a third category, unknown.
- It is the most important category because is an opportunity to open our mind to new knowledge.
- "The supreme goal of all theory is to make the irreducible basic elements as simple and as few as possible without having to surrender the adequate representation of a single datum of experience."

Albert Einstein
Philosophy of Science
Vol. 1, No. 2 (Apr., 1934), pp. 163-169

Márcio Flávio Dutra de Moraes (Brazil)

HYPEREXCITABLE AND HYPER SYNCHRONOUS NEURAL NETWORKS IN ANIMAL MODELS OF EPILEPSY: ASPECTS OF THE TEMPORAL DYNAMICS OF NEURAL RECRUITMENT IN EPILEPTOGENESIS

Jaime Carrizosa (Colombia)

TRANSITION IN EPILEPSY

Transition programs: between a successfull or broken connection in epilepsy care

Jaime Carrizosa Moog
University of Antioquia
Child and Adolescent Neurology Service
Pediatric Department

Medellín, Colombia

THE HEALTH OF ADOLESCENTS AND YOUTH IN THE AMERICAS

IMPLEMENTATION OF THE REGIONAL STRATEGY AND PLAN OF ACTION ON ADOLESCENT AND YOUTH HEALTH 2010-2018

Pan American Health Organization

World Health Organization
Americas

THE HEALTH OF ADOLESCENTS AND YOUTH IN THE AMERICAS

Young persons living with chronic conditions: These young persons have the same developmental issues, challenges, and needs as their peers, in addition to dealing with their chronic condition. While information is limited on the burden of chronic conditions among young persons in the Region, the available data suggest a significant burden, ranging from respiratory conditions such as asthma to diabetes, cancers, epilepsy, skin and musculoskeletal conditions, and HIV. Each

THE HEALTH OF ADOLESCENTS AND YOUTH IN THE AMERICAS

to diabetes, cancers, epilepsy, skin and musculoskeletal conditions, and HIV. Each year in the Americas, more than 600 adolescents aged 10-19 die from epilepsy and more than 1,000 from diabetes and heart disease *(39)*. In an analysis of data

Chronic conditions can be challenging in any stage of life, but the physiological and psychosocial dimensions of adolescence bring added difficulties. Chronic illness during puberty may cause temporary or permanent delays in growth and development, as well as impair the psychosocial development of the young person *(70, 71)*. Some studies have found that young persons with chronic conditions are at higher risk for depression and self-harm, including suicidal ideation and attempts *(72)*.

THE HEALTH OF ADOLESCENTS AND YOUTH IN THE AMERICAS

Another important aspect of chronic disease management in adolescents is the effective transfer of the adolescent from pediatric to adult care. This can take place in early adolescence, depending on the legislation and the health system policies and practices in the country. Careful consideration and attention must be given to facilitate an effective transition, ensuring appropriate support for the adolescent *(73)*.

Figure II.10: Changes in causes of adolescent disability-adjusted life years (DALYs) lost in those 10-14 years old (both sexes), Latin America and the Caribbean, 1990-2015

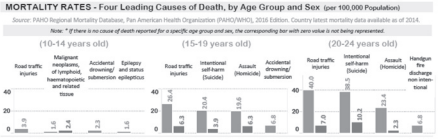
Source: Analysis of data from the 2015 Global Burden of Disease (GBD) study conducted for PAHO by the Institute for Health Metrics and Evaluation (IHME).

Figure II.11: Changes in causes of adolescent disability-adjusted life years (DALYs) lost in those 15-19 years old (both sexes), Latin America and the Caribbean, 1990-2015

Figure II.12: Changes in causes of youth disability-adjusted life years (DALYs) lost in those 20-24 years old (both sexes), Latin America and the Caribbean, 1990-2015

Adolescent and Youth Health - 2017 Country Profile

URUGUAY

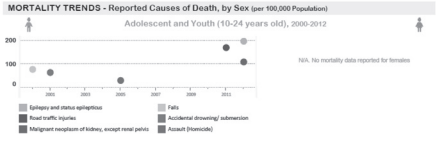


Annex II.B2: Leading causes of death in adolescents (aged 10-19 years) in the Americas in 2011 (39 countries reporting), with number of deaths and age-adjusted rates per 100,000, by sex

Rank	Cause of death	Males		Females		Total	
		Number	Adjusted rate	Number	Adjusted rate	Number	Adjusted rate
1	Assault (homicide)	17,464	22.21	2,119	2.82	19,583	12.73
2	Road traffic injuries	10,042	12.80	3,509	4.67	13,551	8.81
3	Intentional self-harm (suicide)	4,230	5.39	1,910	2.54	6,140	4.00
4	Event of undetermined intent	3,199	4.32	587	0.83	3,786	2.62
5	Malignant neoplasm of lymphoid, hematopoietic and related tissue	1,775	2.27	1,280	1.72	3,055	2.00
6	Accidental drowning and sub-mersion	2,336	3.04	448	0.61	2,784	1.84
7	Congenital malformations, deformations and chromosomal abnormalities	984	1.27	763	1.03	1,747	1.15
8	Influenza and Pneumonia	880	1.13	706	0.94	1,586	1.05
9	Accidental poisoning	778	1.05	373	0.52	1,151	0.79
10	Diseases of the urinary system	556	0.71	454	0.6	1,010	0.66
11	Cerebrovascular diseases	499	0.64	382	0.51	881	0.58
12	Pregnancy, childbirth and the puerperium	0	0.00	830	1.14	830	0.56
13	Malignant neoplasm of brain	453	0.59	320	0.44	773	0.51
14	Septicemia	385	0.52	289	0.40	674	0.45
15	Epilepsy and status epilepticus	396	0.52	257	0.34	653	0.43
16	Others	14,468		9,130		23,598	
	Total	58,445	74.46	23,357	31.11	81,802	53.24

Adolescent and Youth Health - 2017 Country Profile

MONTSERRAT



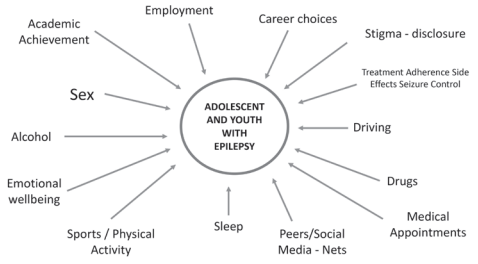
Prevalence Rate of Epilepsy in Latin America

Country	Nr. Studies	Year	Prevalence Rate / 1000
Argentina	3	1991	3,2 – 6,2
Bolivia	2	1994 - 2010	6,6 – 12,3
Brazil	5	2000 - 2006	5,4 – 20,4
Chile	2	1975 - 1988	17,7 – 31,9
Colombia	4	1993 – 2005	10,1 – 24,0
Cuba	1	?	7,5
Ecuador	5	1992 - 2015	7,14 – 28,0
Guatemala	2	1990	8,5 – 28,0
Honduras	2	1997 - 2005	11,8 – 23,4
Mexico	2	2007 - 2010	3,9 – 25,4
Peru	3	2000 – 2007	10,8 – 32,1
Panama	1	?	57
Uruguay	1	1990	9,1
TOTAL	33	MEAN	12,2 – 25,3

Estimated number of young persons (10 – 24 years) with epilepsy in Latin America

2.022.821 – 4.194.866

Rev Neurol 2018; 67: 249-62.



Dynamic mapping of human cortical development during childhood through early adulthood

Nitin Gogtay[1,2], Jay N. Giedd*, Leslie Lusk*, Kiralee M. Hayashi*, Deanna Greenstein*, A. Catherine Vaituzis*, Tom F. Nugent III*, David H. Herman*, Liv S. Clasen*, Arthur W. Toga*, Judith L. Rapoport*, and Paul M. Thompson[2]

*Child Psychiatry Branch, National Institutes of Mental Health, National Institutes of Health, Bethesda, MD 20892; and [1]Laboratory of Neuro Imaging, Department of Neurology, University of California School of Medicine, Los Angeles, CA 90095-1769

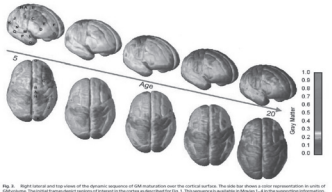
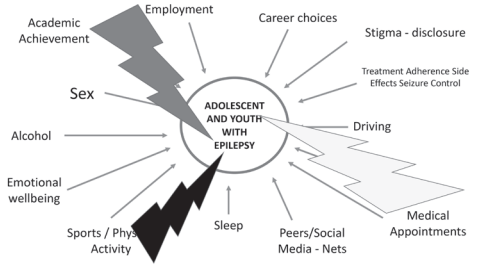


Fig. 3. Right lateral and top views of the dynamic sequence of GM maturation over the cortical surface. The side bar shows a color representation in units of GM volume. The initial frames depict regions of interest in the cortex as described in Fig. 1. This sequence is available in Movies 1–4 in the supporting information.



Epilepsy & Behavior 56 (2016) 1–4

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Early sexual debut in Norwegian youth with epilepsy: A population-based study

Morten I. Lossius [a,*], Kristin Å. Alfstad [a], Betty Van Roy [b], Petter Mowinckel [c], Jocelyne Clench-Aas [d], Leif Gjerstad [e,f], Karl O. Nakken [a]

A B S T R A C T

Objectives: In comparison with controls, youth with epilepsy (YWE) have greater psychosocial problems. However, information about their sexual behavior is sparse. We have performed a large, population-based questionnaire study to examine differences in sexual behavior between YWE and controls.

Methods: A randomly chosen cohort of youth (13–19 years) from Akershus county, Norway (n = 19,995) was asked to complete a questionnaire anonymously with questions on epilepsy and sexual activity.

Results: The response rate was 85%. Two hundred forty-seven participants reported having or having had epilepsy, i.e., a lifetime epilepsy prevalence of 1.2%. Compared with controls, a higher proportion of YWE reported having had sexual intercourse (43.6% vs. 35.3%, p = 0.009). The mean age at sexual debut was significantly lower in YWE than in controls (14.0 years vs. 15.0 years, p < 0.001), and this was particularly marked among boys. A higher proportion of YWE reported not having used contraceptives at their last sexual intercourse compared with controls (31.6% vs. 22.3%, p = 0.03). Ten percent of YWE, compared with 2% of the controls, reported that they had been forced into their first sexual intercourse.

Conclusion: In YWE, some aspects of sexual behavior differ from those of their peers, with earlier sexual debut and less frequent use of contraceptives. More attention should be directed toward this subject, aiming at avoiding unwanted pregnancies and potential emotional traumas in this already vulnerable patient group.

Seizure 57 (2018) 50–55

Contents lists available at ScienceDirect

Seizure

journal homepage: www.elsevier.com/locate/yseiz

Perceived stigma and associated factors among children and adolescents with epilepsy in south western Uganda: A cross sectional study

Joseph Kirabira [a,*], Madrine Nakawuki [a], Robyn Fallen [b], Godfrey Zari Rukundo [a]

A B S T R A C T

Purpose: To determine the prevalence of perceived stigma and its associated factors among children and adolescents with epilepsy in southwestern Uganda.

Methods: We conducted a cross sectional study at a large referral hospital and a small rural health facility in Mbarara district, southwestern Uganda. Participants were aged 6–18 years being managed for epilepsy for at least 3 months, with no medical emergencies. Perceived stigma was measured using the Kilifi Stigma Scale of Epilepsy. Data on associated factors were collected by a pre-piloted investigator designed questionnaire. Logistic regression was used to determine associated factors considering 5% statistical significance.

Results: Prevalence of high perceived stigma was 34% with higher levels among older children and adolescents. Children who had never attended school were more likely to report perceived stigma (62%). Factors associated with this stigma included having epilepsy related injuries or deformities (p = 0.022), other chronic illnesses (p = 0.009) and a longer duration of antiepileptic drug use (p = 0.004).

Conclusions: Perceived stigma of epilepsy remains a major public health problem among children and adolescents and it is highly associated with preventable or modifiable factors. Therefore, there is need to design interventions that can address these factors in order to reduce the stigma and its potential future complications such as educational inequalities.

Epilepsia, **47**(3):631–639, 2006
Blackwell Publishing, Inc.

Physical Activity in Children/Teens with Epilepsy Compared with That in Their Siblings without Epilepsy

*Judy Wong and †Elaine Wirrell

**University of Alberta, Edmonton; and †Department of Pediatrics and Neurosciences, University of Calgary, Calgary, Alberta, Canada*

Summary: *Purpose:* To determine (a) whether children and teens with epilepsy participate in less physical activity and have higher body mass index (BMI) percentiles for age than do their siblings without epilepsy; and (b) what epilepsy-specific factors limit their participation.

Methods: Patients 5–17 years, with a ≥3 month history of epilepsy, a development quotient ≥80, no major motor or sensory impairments, and at least one sibling without epilepsy in a similar age range, were identified from the Neurology Clinic database or at the time of clinic visit. Parents completed a questionnaire regarding sedentary activities and group, individual, and total sports activities. Children aged 11–15 years also completed the physical activity portion of the Health Behavior in School Aged Children questionnaire. Clinic charts were reviewed for seizure type, etiology, frequency, duration of epilepsy, and number of antiepileptic drugs (AEDs) ever taken.

Results: Teens with epilepsy participated in fewer group and total sports activities than did controls and were more likely to be potentially overweight or overweight. Receiving three or more AEDs in the past showed a significant negative correlation with sports participation. Although a trend was noted for those with higher seizure frequency to be less active, no other epilepsy-specific factors or prior seizures or seizure-related injury during a sports event correlated with participation in physical activity.

Conclusions: Programs that promote exercise in adolescents with epilepsy should be encouraged to improve their physical, psychological, and social well-being. **Key Words**: Physical activity—Sports—Overweight.

Epilepsy Research 136 (2017) 35–45

Contents lists available at ScienceDirect

Epilepsy Research

journal homepage: www.elsevier.com/locate/epilepsyres

Review article

The impact of epilepsy on academic achievement in children with normal intelligence and without major comorbidities: A systematic review

S.W. Wo[a], L.C. Ong[b], W.Y. Low[c], P.S.M. Lai[a,*]

A B S T R A C T

Purpose: To systematically examine published literature which assessed the prevalence of academic difficulties in children with epilepsy (CWE) of normal intelligence, and its associating factors.

Methods: A search was conducted on five databases for articles published in English from 1980 till March 2015. Included were studies who recruited children (aged 5–18 years), with a diagnosis or newly/recurrent epilepsy, an intelligent quotient (IQ) of ≥ 70 or attending regular school, with or without a control group, which measured academic achievement using a standardised objective measure, and published in English. Excluded were children with learning difficulties, intellectual disabilities (IQ < 70) and other comorbidities such as attention deficits hyperactive disorder or autism. Two pairs of reviewers extracted the data, and met to resolve any differences from the data extraction process.

Results: Twenty studies were included. The majority of the studies assessed "low achievement" whist only two studies used the IQ-achievement discrepancy definition of "underachievement". Fourteen studies (70%) reported that CWE had significantly lower academic achievement scores compared to healthy controls, children with asthma or reported norms. The remaining six studies (30%) did not report any differences. CWE had stable academic achievement scores over time (2–4 years), even among those whose seizure frequency improved. Higher parental education and children with higher IQ, and had better attention or had a positive attitude towards epilepsy, were associated with higher academic achievement score. Older children were found to have lower academic achievement score.

Conclusions: In CWE of normal intelligence, the majority of published literature found that academic achievement was lower than controls or reported norms. The high percentages of low achievement in CWE, especially in the older age group, and the stability of scores even as seizure frequency improved, highlights the need for early screening of learning problems, and continued surveillance.

Psychiatric and Behavioural Disorders in Children with Epilepsy (ILAE Task Force Report)
Epileptic Disord 2016; 18 (Suppl. 1): S2-S7

Psychiatric and Behavioural Disorders in Children with Epilepsy (ILAE Task Force Report): Epidemiology of psychiatric/behavioural disorder in children with epilepsy*

Matti Sillanpää[1], Frank Besag[2], Albert Aldenkamp[3], Rochelle Caplan[4], David W. Dunn[5], Giuseppe Gobbi[6]

High comorbidity: ADHD (30%), Cognitive disability (40%), Anxiety (15-36%), Depression (8-35%), Autism (20%), Psicosis (2%).

Epileptic Disorders (2016), Vol. 18, Supplement 1; Developmental Medicine Child Neurology(1999), 41, 473–479; Epilepsia (2014), 55(12):1910–1917.

Epilepsy & Behavior 78 (2018) 104–108
Contents lists available at ScienceDirect
Epilepsy & Behavior
journal homepage: www.elsevier.com/locate/yebeh

The "Cinderella Syndrome": A narrative study of social curfews and lifestyle restrictions in juvenile myoclonic epilepsy

Teresa Leahy, Michael J. Hennessy, Timothy J. Counihan *

ABSTRACT

Several factors are thought to contribute to inadequate seizure control in patients with juvenile myoclonic epilepsy (JME), including drug resistance, neuropsychiatric comorbidity, and poor lifestyle choices. Recent evidence supports the existence of frontal lobe microstructural deficits and behavioral changes that may contribute to poor seizure control in a minority of patients. Counseling patients on the importance of adequate sleep hygiene and alcohol restriction is an important part of the management strategy for patients with JME. However, information is lacking on how these lifestyle restrictions impact on patients with JME. We conducted a qualitative descriptive analysis of the social impact of JME on 12 patients, from their own perspective. We identified four prominent themes: the importance of alcohol use as a social "norm", how JME affected relationships, decision making (risk versus consequences), and knowledge imparting control. Given that these restrictions were interpreted by patients as social "curfews", we suggest that the term "Cinderella Syndrome" encapsulates the perceived imperative to be home before midnight. Our findings underscore the importance for clinicians to recognize that in counseling patients with JME about lifestyle adjustments, there may be a significant social consequence unique to this patient group.

Determinants of Social Outcomes in Adults With Childhood-onset Epilepsy

Anne T. Berg, PhD,[a,b] Christine B. Baca, MD, MSHS,[c,d] Karen Rychlik, MS,[e] Barbara G. Vickrey, MD, MPH,[f] Rochelle Caplan, MD,[d] Francine M. Testa, MD,[h,i] Susan R. Levy, MD[h,j]

To cite: Berg AT, Baca CB, Rychlik K, et al. Determinants of Social Outcomes in Adults With Childhood-onset Epilepsy. *Pediatrics*. 2016;137(4):e20153844

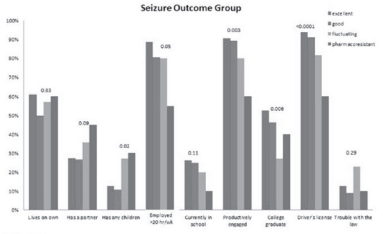


FIGURE 2
Association between seizure course and each of the benchmark social outcomes studied.

Seizure 46 (2017) 19–23

Contents lists available at ScienceDirect

Seizure

journal homepage: www.elsevier.com/locate/yseiz

Epidemiological study of mortality in epilepsy in a Spanish population

María Isabel Chamorro-Muñoz[a,*], Guillermina García-Martín[a], Francisco Pérez-Errazquin[a], Manuel Romero-Acebal[a], Antonio García-Rodríguez[b], Mario Gutiérrez-Bedmar[b]

ABSTRACT

Purpose: Studies concerning mortality in epilepsy have been performed primarily in Northern-Central Europe and US. The aim of this study was to provide information about mortality in people with epilepsy in Southern European countries.

Method: We studied a Spanish prevalence and incidence cohort of 2309 patients aged ≥14 years with epilepsy who were treated in an outpatient epilepsy clinic between 2000 and 2013. The deceased were identified through Civil Registries. Causes of death were determined using death certificates, forensic autopsies, hospital reports, family practitioners, and care-givers' records. Standardised mortality ratios (SMRs) were calculated.

Results: In a total of 15,865 person-years of follow-up, 152 patients died, resulting in an SMR of 2.11 (95% CI 1.79–2.47), which was higher for those aged 14–24. There was also a high rate of death for symptomatic epilepsies, progressive causes (SMR = 6.12, CI 3.50–9.94), and remote causes (SMR = 2.62, CI 2.12–3.21). High SMRs were found for all kinds of epilepsy and for respiratory and tumoural causes. Patients who died of epilepsy itself were 12.5%. Sudden unexpected death in epilepsy incidence was 0.44:1000. Death from status epilepticus incidence was 20:100,000. SMRs for external causes were of no statistical significance.

Conclusions: This is the first epidemiological study to examine rate of mortality in epilepsy in a Southern European country. The identified mortality pattern is similar to the one provided by researchers from developed countries. The similarities between our results concerning epilepsy-related deaths and those provided by population-based studies are the result of the scarcely selected character of our study cohort.

Epilepsy & Behavior 57 (2016) 161–166

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Hospital care for mental health and substance abuse in children with epilepsy

Dylan P. Thibault [a,b], Adys Mendizabal [c], Nicholas S. Abend [a,d], Kathryn A. Davis [a,e], James Crispo [b,f], Allison W. Willis [a,b,g,h,*]

A B S T R A C T

Results: We observed 353,319 weighted MHSA hospitalizations of children ages 6–20; 3280 of these involved a child with epilepsy. Depression was the most common MHSA diagnosis in the general population (39.5%) whereas bipolar disorder was the most common MHSA diagnosis among children with epilepsy (36.2%). Multivariate logistic regression models revealed that children with comorbid epilepsy had greater adjusted odds of bipolar disorder (AOR: 1.17, 1.04–1.30), psychosis (AOR: 1.78, 1.51–2.09), sleep disorder (AOR: 5.90, 1.90–18.34), and suicide attempt/ideation (AOR: 3.20, 1.46–6.99) compared to the general MHSA inpatient population. Epilepsy was associated with a greater LOS and a higher adjusted incidence rate ratio (IRR) for prolonged LOS (IRR: 1.12, 1.09–1.17), particularly for suicide attempt/ideation (IRR: 3.74, 1.68–8.34).

CRITICAL REVIEW AND INVITED COMMENTARY

Epilepsy: Transition from pediatric to adult care. Recommendations of the Ontario epilepsy implementation task force

[1*]Danielle M. Andrade, [2]Anne S. Bassett, [3]Eduard Bercovici, [4]Felipe Borlot, [5]Esther Bui, [3*]Peter Carrfield, [6]Guida Quaglia Clozza, [7]Eyal Cohen, [8]Timothy Gofine, [9]Lisa Graves, [10]Jon Greenaway, [11*]Beverly Guttman, [12]Maya Guttman-Slater, [13]Ayman Hassan, [14]Megan Henze, [15]Miriam Kaufman, [16]Bernard Lawless, [17]Hannah Lee, [18]Leslee Lindzon, [19]Lysa Boissé Lomax, [20]Mary Pat McAndrews, [21]Dolly Menna-Dack, [21,22]Berge A. Minassian, [23]Janice Mulligan, [24]Rima Nabbout, [25]Tracy Nojin, [26]Mary Secco, [27]Laurens Sellers, [28]Michelle Shapiro, [29]Marie Sieger, [30]Rosie Smith, [31]Peter Szatmari, [32]Leeping Tao, [33]Anastasia Vogt, [34]Sharon Whiting, and [35]O. Carter Snead III

Epilepsia, 58(9):1502–1517, 2017
doi: 10.1111/epi.13832

Transition:

Transition of care is the planned, coordinated movement of adolescents from the child – oriented family centered environment of pediatrics to the adult oriented care setting. (Process)

Transition is the integral** empowerment* of patients/families with a chronic disease in a planned, coordinated movement of adolescents from the child – oriented family centered environment of pediatrics to the adult oriented care setting. (Process)

Transition:

* Empowerment:

Authority or power given to someone to do something. The philosophy of health promotion is to guide and support patient care through empowerment and collaboration.

**Integral:

1. Diagnosis and management of seizures
2. Mental health and psychosocial issues
3. Financial, community and legal supports

Transfer:

The action of handing over a patient to an adult health care provider. (Step)

Step 1: Preparation for the adult health care system

- Introduce the concept of transition.
- Education about "your own disease".
- Need for developing knowledge and skills to be more independent.
- Search services for adults with disabilities.
- Age of discharge.

INTRODUCTORY PHASE (AGE 12 – 15 YEARS)

Step 1: Preparation for the adult health care system

- Develop an individual transition plan
- Provide specific information to individual needs/risks/interests/concerns
- Empowerment of care

PREPARATION PHASE (AGE 12 – 17 YEARS)

Step 1: Preparation for the adult health care system

- Where possible the pediatric neurologist/epileptologist and their team consult with the patient and familiy along with the adult neurologist/epileptologist.
- Follow – up or evaluation of the whole transition process

FINAL PHASE OF TRANSITION (AGE 17 - 18 YEARS)

Step 2. Identifying adolescents at risk for poor transition

- Inconsistent medication compliance
- Risk of unwanted pregrancy
- Use of recreational /illegal drugs
- Driving and seizures
- Comorbidity

- Identify knowledge gaps

Step 3: Epilepsy reevaluation, screening and management

- Epilepsy diagnosis reevaluation
- EEG or videoEEG monitoring
- Imaging
- Genetic testing
- Mental health needs/screening
- Treatment: neuromodulation, ketogenic diet
- Contraception and family planning
- Healthy lifestyle: nutrition, physical activity, sleep

Step 3: Epilepsy reevaluation, screening and management

- Epilepsy diagnosis reevaluation: West Syndrome → LGS → Focal epilepsy

- EEG or videoEEG monitoring:

  - Patient is not seizure free.
  - Seizure semiology has changed
  - Patient has other non epileptic events
  - Possible psychologic non epileptic events (PNES)
  - Adult service without easy availability of EEG service

Step 3: Epilepsy reevaluation, screening and management

- Imaging:

  - If an MRI has never been done (?)
  - Progressive etiology
  - Seizure change in semiology or frequency
  - Patient as posible surgery candidate

Step 3: Epilepsy reevaluation, screening and management

- Genetic Testing:

  - Chromosome microarray, massive parallel sequencing, exome or genome sequencing
  - Possibility that adult neurologist is (more?) unfamiliar with genetic testing

Step 3: Epilepsy reevaluation, screening and management

- Mental health needs:
  - Mental health screening (Depression, anxiety, suicidal ideation, ADHD)
  - Bipolar disorder and schizophrenia beginning in adolescence
  - Intelectual dissability reevaluation

  Questionnaires for normal adolescents
  - GAINS (Global Appraisal of Individual Needs Short Sceener)
  - MFQ (Mood and Feelings Questionnaire)
  - THRxEADS (Transition, Home, Medication, Education, Activity, Peers, Drugs, Suicidality, Body Image)
  - HEADSS (Home, Education, Employment, Activities, Drugs, Sexuality, Suicidality, Depression)
  - ADOLESCENTE

FACULTAD DE MEDICINA - UNIVERSIDAD DE ANTIOQUIA
PROGRAMA: MEDICINA
CURSO: ADOLESCENCIA Y (SEBETIO).
ENCUESTA ADOLESCENTES TRANSICIÓN
Departamento de Pediatría y Puericultura

	VARIABLE - ÍNDICES
A	**Ánimo. Armas. Automóviles** Ansiedad, depresión, temperamento, conductas disruptivas, impulsividad, hiperactividad, niñas, autoestima; motos, carros, licencia de conducción; arma blanca o de fuego
D	**Drogas** Conocimiento y prevención, acceso, exposición, consumo, presión de grupo, actitud en la familia, acceso a consulta
O	**Ocupación. Ocio. Esparcimiento. hobby** Estudio, trabajo, expectativa laboral, tiempo libre, vínculo a grupos juveniles y comunitarios
L	**Licor** Conocimiento y prevención, acceso, exposición, consumo, presión de grupo, actitud en la familia, acceso a consulta
E	**Enfermedades** Conocimiento y mecanismos de adaptación (actos impulsivos, proyección, pánico, regresión, negación, separación, distanciamiento, aislamiento, aceptación, intelectualización, compensación,) adherencia terapéutica; efecto deseado y efectos secundarios, interacción farmacológica y con otras sustancias, controles médicos, pronóstico
S	**Salud. Sueño. Suicidio** Controles médicos, vacunación, tamización; hábitos de sueño, horarios, efectividad, ideación suicida
C	**Casa** Composición, relacionamiento, identificación de riesgos, ingresos etc.
E	**Ejercicio. Actividad física** Tipo, regularidad, intensidad, expectativa, ventajas, desventajas y ventajas
N	**Nutrición** Hábitos, estado nutricional
T	**Televisión y Redes Sociales** Hábitos, riesgos, ventajas, horas/día, tipo de videojuegos, género de programas
E	**Educación. Expectativas** Aprendizaje, cursos, *bullying* expectativa laboral
S	**Sexualidad** Conocimiento, ETS, ACO, IVE, riesgos, violencia sexual

Step 3: Epilepsy reevaluation, screening and management

- Treatment: neuromodulation, ketogenic diet

- Contraception and family planning

  - Drug interactions
  - Genetic counseling
  - Folate/iron supplementation
  - Teratogenicity

- Healthy lifestyle: nutrition, physical activity, sleep, "virtual media" addiction

Step 4: Community, social, financial and legal support

✓ Constitutional and legal rights
✓ Disability rights
✓ Right for health assistance and education
✓ Social security and other kind of insurances
✓ Support groups
✓ Vocational/professional orientation
✓ Epilepsy education for friends, relatives, employers

Step 5: Involvement of the family physician

Step 6: Pediatric discharge package

✓ Transition readiness questionnaire
✓ Complete medical history
✓ Referrals
✓ Goals of care
✓ Community, social, financial and legal support

V. APPENDIX 2: Transition Readiness Checklists

For the person with Epilepsy

Name of Patient:_____ Name of Institution:_____
Age:_____ Health Condition:_____
Gender:_____ Date:
Do you have a Family Physician? ❑ Yes ❑ No
If yes please list name:_____

	For each of the following statements please select the response that best suits you	No, I do not know	No, but I am learning to do this	Yes, I have started doing this	Yes, I always do this	Does not apply to me
1	I can describe my health condition and explain my health care needs to others					
2	I know what triggers my seizures and how to minimize the triggers					
3	I know what to do in the event of a medical emergency relating to my condition (first aid; when to call 911)					
4	I know how to call the doctor about unusual changes in my health (for example: medication side effects)					
5	I know the names of the medications I take					
6	I know how to take medications correctly on my own and have a system in place to remind me when to take them					
7	I know when and how to reorder medications before they run out					
8	I have had a discussion about how certain medications can impact birth control and pregnancy					
9	I can call my doctor's office to make or change an appointment					
10	I make a list of questions to ask my doctor before going to appointments					

	For each of the following statements please select the response that best suits you	No, I do not know	No, but I am learning to do this	Yes, I have started doing this	Yes, I always do this	Does not apply to me
11	I organize and keep track of my health information (appointments, medications, seizures, etc.)					
12	I can get to medical appointments on my own					
13	I spend time alone with my health care provider at each appointment					
14	I speak up for myself and tell others what I need during health care visits					
15	I have discussed sexuality and reproductive health with my health care team (consent/ sexually transmitted infections/contraception)					
16	I know how my lifestyle can impact my health condition and how to discuss this with my health care team (e.g. use of alcohol, drugs, lack of sleep etc.)					
17	I understand the rules and regulations about epilepsy and driving					
18	I understand the implications of my heath condition on career choice and future employment					
19	I know my legal rights as a person living with this health condition and how to access necessary accommodations at school and at work					
20	I know about my health insurance coverage. If on parents plan currently, I know the plan for coverage when my parent(s) health insurance runs out					
21	I know about my right to privacy, confidentiality and decisions-making regarding my health.					
22	If I chose to, I know how to disclose my epilepsy to friends, classmates, coworkers and others					
23	I know how to access the supports I need if I feel stressed, depressed or anxious					
24	I know what to expect in adult services and how it differs from paediatric services					

TRANSITION OF EPILEPSY CARE FROM CHILDREN TO ADULTS

Models for transition clinics

*Jaime Carrizosa, †Isabelle An, ‡Richard Appleton, §Peter Camfield, and ¶Arpad Von Moers

Epilepsia, 55(Suppl.3):46–51, 2014
doi: 10.1111/epi.12716

Table 2. Proposed transition checklist

Name:
Age:
Diagnosis:
Treating physician:
Receiving physician:

Transition items – date	Key notes	Observations
Complete medical history	Diagnosis and comorbidity; medication schedule(s) adherence, AED efficacy, drug interactions, adverse effects, AED withdrawal trials, plasma concentrations, seizure follow-up diaries; neuroimaging, electrophysiologic, neuropsychological studies; liver function, blood tests	
Education information	Building up awareness and consciousness about his/her disease, drug treatment, triggering factors; stigma; legal rights	
Family dynamics	Quality of parent adolescent relationship, overprotection, dependence, shame, cultural and religious background, resilience	
Individual health supervision issues	Nutritional status: weight/height/BMI; vaccinations, body image	
Sexuality, pregnancy and reproductive issues	Anticonception, irregular menses, sexual performance and desire, sexually transmitted diseases, pregnancy risks, sexual abuse, teratogenesis, pregnancy termination, breastfeeding	
Smoking, alcohol, drugs	Seizure risks, antiepileptic drug interaction, dependence, addiction, peer pressure	
Education and career choices	Vocational, technical, professional orientation; overnight duties	
Physical activity	Extreme sports, seizure control, SUDEP prevention	
Driver's license	Legislation, seizure control, autonomy	
Comorbidity	Physical and psychiatric comorbidity, drug treatment and interactions, quality of life	
Mortality	Higher risk of mortality, SUDEP, depression, suicide, accidents	
Insurance	Social security, family dependence, work insurance, treatment and follow-up guarantee	

Accepted Manuscript

Title: Failed transition to independence in young adults with epilepsy: The role of loneliness

Authors: R.P.J. Geerlings, L.M.C. Gottmer-Welschen, J.E.M. Machielse, A.J.A. de Louw, A.P. Aldenkamp

CHALLENGES

- ✓ Geographical distribution of clinical settings
- ✓ Adult neurologists dealing with pediatric disorders (diagnosis, treatments, comorbidities)
- ✓ Costs of the transition strategy
- ✓ Follow up after transfer – How to measure the impact?
- ✓ Where is the balance between adolescents´ autonomy/independence and the risk of medicalization of normal develpmental processes or overprotection (fear of losing control over patients)?

GROUP C – CASE STUDY

GROUP A – CASE STUDY

GROUP B – CASE STUDY

GROUP D – HOW TO KEEP AND REPORT YOUR RESEARCH ACTIVITY?

Ferruccio Panzica (Italy)

IDENTIFICATION OF EPILEPTIC ZONE FROM STEREOEEG: A CONNECTIVITY BASED APPROACH

Identification of epileptic zone from StereoEEG:
a connectivity based approach

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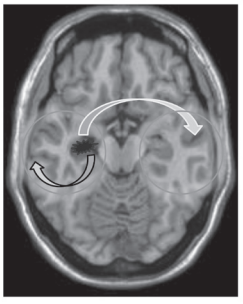
Focal seizures

- Originate within networks limited to one hemisphere
- May be discretely localized or more widely distributed….



Scheffer ILAE 2017

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Focal seizures

- Focal epilepsies represent a common neurological disorder and account for more than 50% of all epilepsies.
- Approximately 30% of patients with focal epilepsies experience seizures that are resistant to anti-epileptic drugs
- A subset of these patients can be considered candidates for epilepsy surgery
- The main issue to be solved is the precise definition of *the Epileptogenic Zone (EZ)*

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Epileptogenic Zone


LA
STÉRÉO-ÉLECTROENCÉPHALOGRAPHIE
DANS L'ÉPILEPSIE
INFORMATIONS NEUROPHYSIOPATHOLOGIQUES
APPORTÉES PAR
L'INVESTIGATION FONCTIONNELLE STÉRÉOTAXIQUE
PAR
J. BANCAUD, J. TALAIRACH
ET
A. BOZIER, G. SCHAUB, G. SERDA, P. MORGE, M. BONIS-DARCOS
M
MASSON & Cie

In 1965, Talairach and Bancaud introduced the term "epileptogenic zone" (EZ) as "the site of the beginning and of their primary organization of the epileptic seizures "

EZ the "area of cortex that is necessary and sufficient for initiating seizures and whose removal (or disconnection) is necessary for complete abolition of seizures (Luders, 1992)

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Indications for Stereo-EEG in drug-resistant focal epilepsies

- Discrepancies between:
  - ✓ anatomical and clinical data;
  - ✓ anatomical and electrical data;
  - ✓ electrical and clinical data;
- Supposed bi- or multi-lobar ictal discharges;
- Early ictal involvement of functional cortical areas (suggested by the clinical semiology);

⇩

Individually tailored surgical removal

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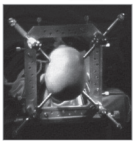
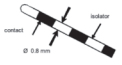
Stereo-EEG.

Diagnostic invasive technique used for pre-surgical evaluation of patients with drug-resistant partial epilepsy.
It consist in stereotactic placement of intracerebral multilead electrodes for EEG recordings. 150-200 EEG signals are recorded for 1-2 weeks, and analysed in order to correctly identify the Epileptogenic Zone





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The appropriate identification of the EZ, is the fundamental step in the diagnostic work-up prior to surgery.

Its organization can be quite complex: the seizure onset may involve distant and functionally distinct brain sites almost simultaneously

The issue of defining the EZ is closely related to that of identifying 'abnormal' couplings among neuronal ensembles distributed over distant areas.

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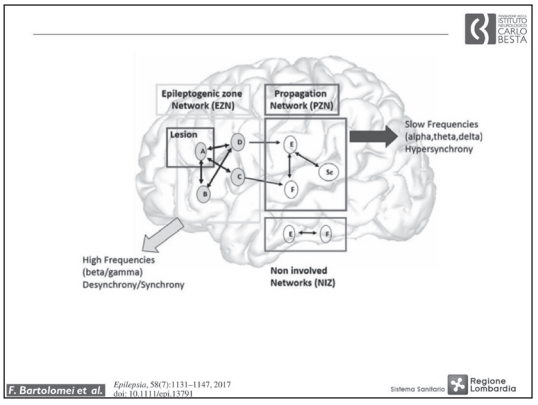

Epileptic Seizure

A transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (ILAE)

- Can abnormal couplings be identified?
- How do coupling and directions evolve during the interictal to ictal transition and the seizure?
- **Do some area play a leading role in the seizure generation process**?




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NeuroImage 61 (2012) 591–598

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NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Epileptogenic networks of type II focal cortical dysplasia: A stereo-EEG study

Giulia Varotto [a,c], Laura Tassi [d], Silvana Franceschetti [a], Roberto Spreafico [b], Ferruccio Panzica [a,*]

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[b] Clinical Epileptology and Experimental Neurophysiology Unit, C. Besta Neurological Institute IRCCS Foundation, Milan, Italy
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The aim of this study was to evaluate the changes in dynamic connectivity pattern under inter-ictal, pre-ictal and ictal conditions using signals derived from stereo-EEG recordings of 10 patients with Taylor-type focal cortical dysplasia.
A causal linear multivariate method – partial directed coherence – and indices derived from graph theory were used to characterise the synchronisation property of the lesional zone (corresponding to the epileptogenic zone in our patients) and to distinguish it from other regions involved in ictal activity or not.

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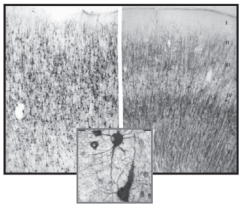
Type II (Taylor's Focal Cortical Dysplasia

Focal Cortical Dysplasias: Cortical malformations characterized by focal anomalies of cortical structures. **They are often associated with drug-resistant epilepsy**

- ✓ loss of cortical lamination (laminar cortical disruption, disorganization of the normal layering of the cortex)
- ✓ Dysmorphing neurons (giant neurons, large ectopic neurones in the white matter
- ✓ +/- Ballon cells



- spatially circumscribed
- intrinsic epileptogenic
- peculiar intrinsic organization
- distinctive interictal activity
- good surgical outcome

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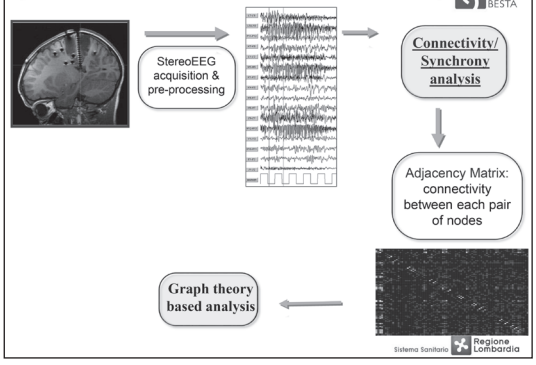
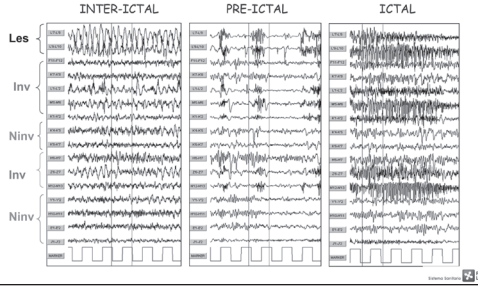
Methodological Procedure



A sub-set of between 17 and 24 bipolar derivations was selected for analysis

- Leads positioned inside the dysplasia= the lesional group (LES);
- outside the lesion showing epileptiform activity during seizures= involved group (INV);
- outside the lesion without any epileptiform activity during seizures= non-involved group (N-INV);



CONNECTIVITY

Functional Connectivity: statistic measure of temporal interaction between activities in different areas.

Effective Connectivity: influence that one neural system exerts on another (who drives whom). It describes the dynamic directional interactions (causal effects) among brain regions

Couplings are:
- ✓ ***transient***
- ✓ ***dynamic*** (time-varying)
- ✓ ***frequency-specific***

Autoregressive Models (AR)

MULTIVARIATE AR

$$\begin{bmatrix} x_1(t) \\ \vdots \\ x_Q(t) \end{bmatrix} = \sum_{k=1}^{p} A_k \begin{bmatrix} x_1(t-k) \\ \vdots \\ x_Q(t-k) \end{bmatrix} + \begin{bmatrix} w_1(t) \\ \vdots \\ w_Q(t) \end{bmatrix} \qquad A_k = \begin{bmatrix} a_{1,1}(k) & a_{1,2}(k) & \cdots & \cdots & a_{1,Q}(k) \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots & a_{n,m}(k) & \vdots \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ a_{Q,1}(k) & \cdots & \cdots & \cdots & a_{Q,Q}(k) \end{bmatrix}$$

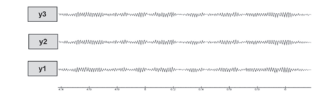
Partial Directed Coherence (PDC)

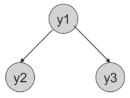
$$\bar{A}(f) = I - A(f) \qquad A(f) = \sum_{k=1}^{p} A_k e^{-2j\pi fk},$$

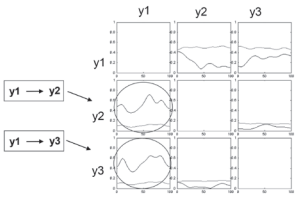
$$Pdc_{ij}(f) = \frac{\bar{a}_{ij}}{\sqrt{\bar{a}_j^{\,H}(f) \cdot \bar{a}_j(f)}}$$

$$\bar{\mathbf{A}}(f) = \begin{bmatrix} \bar{a}_{11}(f) & \cdots & \bar{a}_{1j}(f) & \cdots & \bar{a}_{1m}(f) \\ \bar{a}_{21}(f) & \ddots & \vdots & & \vdots \\ \vdots & & \bar{a}_{ij}(f) & & \vdots \\ \vdots & & \vdots & \ddots & \vdots \\ \bar{a}_{m1}(f) & \cdots & \bar{a}_{mj}(f) & \cdots & \bar{a}_{mm}(f) \end{bmatrix}$$

PDC





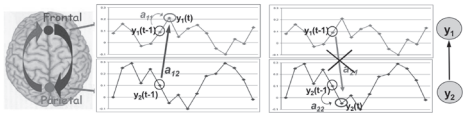


- Takes into account all the signals
- Can distinguish between direct and indirect connections.
- Can avoid the common source effect.

— PDC Spectra — Statistical Threshold

Granger Causality

A process X_t is said to Granger cause another process Y_t if future values of Y_t can be predicted better using past values of X_t and Y_t than using the past of Y_t alone.



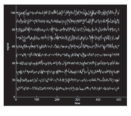
$$y_1(t) = a_{11}y_1(t-1) + \boxed{a_{12}}y_2(t-1) + e_1(t)$$
$$y_2(t) = \boxed{a_{21}}y_1(t-1) + a_{22}y_2(t-1) + e_2(t)$$

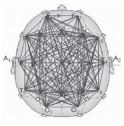
Granger Causality $a_{12} \neq 0$ Granger Non Causality $a_{21} = 0$

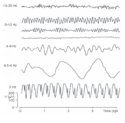
A network is a mathematical representation of a real-world complex system and is defined by a collection of ***nodes (vertices)*** and ***links (edges)*** between pairs of nodes.

Nodes in large-scale brain networks usually represent brain regions, while links represent anatomical, functional, or effective connections depending on the dataset

Networks → graph analysis







Undirected graph with n vertices: maximum No. of edges = n(n-1)/2
Directed graph with n vertices: maximum No. of edges = n(n-1)

EEG: 19 channels → 342 measures (edges)
HDEEG: 128 channels → 16256 edges
MEG: 306 channels → 93330 edges

Huge amount of data. Difficult to extract information

Graph Theory Approach

Centrality measures

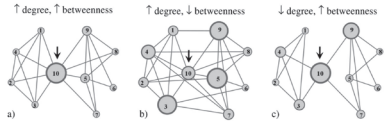
How important is a node in a network?

1. Degree centrality

Important nodes have many connections

2. Betweenness centrality

Important nodes work as bridges



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Degree

When ties are directed, we can calculate the ***out-degree=*** total number of links sent and (***in-degree***) = total number of links received

Out-degree indicates influence
in-degree indicates prestige or popularity

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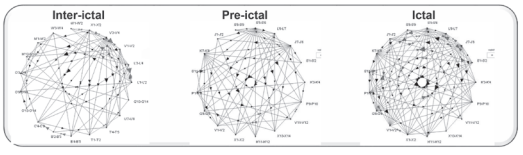

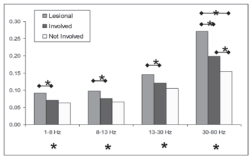

A significantly different connectivity pattern (mainly in the gamma band) distinguishes the EZ from other cortical regions not only during the ictal event, but also during the inter-and pre-ictal periods

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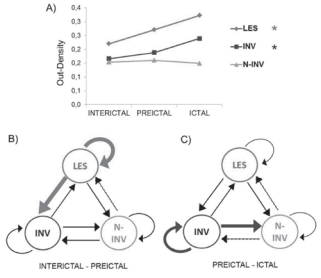
Temporal Dynamic changes



the dysplasia plays a leading role in generating and propagating ictal EEG activity by acting as an hub.
The other cortical regions involved in the ictal activity essentially act as "secondary" generators.

A) Asterisks indicate a significant increase. B) and C): graphic representation of the significant (colored arrows) and non-significant (black arrows) increases between two temporal conditions (Varotto et al., Neuroimage 2012)

Comments

In patients with type II FCD, the region inside the dysplasia corresponds to the abnormal hubs of the epileptic network that originates and sustains the seizures, plays a leading role in generating and propagating ictal EEG activity, and in recruiting other distant areas to become involved in the seizure. This leading role of the dysplasia may account for the good post-surgical outcome of patients with type II FCD because the resection of dysplastic tissue removes the entire EZ responsible for seizure onset.

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Comments

In all of the examined cases, we observed significant differences in the interactions between SEEGs obtained from EZ, other regions involved in the ictal activity and regions non-involved in seizure

Effective connectivity + indexes from graph theory can add new information to support clinician in localizing the EZ (EN)

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European Project (Fp7 funded project)

DESIRE

SEVENTH FRAMEWORK PROGRAMME

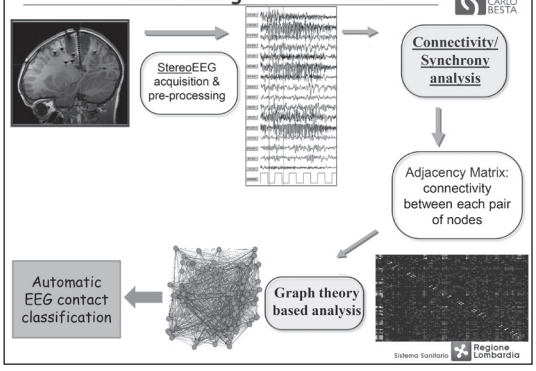
Project full title: " Development and Epilepsy - Strategies for Innovative Research to improve diagnosis, prevention and treatment in children with difficult to treat Epilepsy "

Grant agreement no: 602531

Non-invasive neurophysiologic biomarkers for the recognition of the epileptogenic zone and networks interfering with cognitive brain function in FCD patients

Grant Agreement no: 602531 Sistema Sanitario Regione Lombardia

Methodological Procedure



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Methods

- 14 patients recorded at the Epilepsy Surgery Center of Niguarda Hospital (Milano) with drug-resistant focal epilepsy, all seizure free after one year from surgery.
- Analysis was performed on the entire set of SEEG contacts using a bipolar montage
- And focused on interictal SEEG signals: 3 minutes, divided into 36 consecutive non-overlapping epochs
- For the connectivity analysis we used the h2 index (*Bartolomei et al., 2001*).

- Several graph theory based indexes were used to test different classification procedures.
  Indices characterizing the centrality of a node were better related to the ZE, namely: degree, strength and betweenness centrality

- For an automatic classification of the nodes we used the following procedure:
  For each graph index we defined as threshold the average value among the nodes and time epochs.
  For each graph index, we assigned a value of 1 to a node when its mean value was higher than the threshold, 0 otherwise.
  A Node was classified as belonging to EZ if at least one of the three index had value of 1.
- The same procedure was applied on different frequency bands and the results compared.
- The accuracy of the classification was evaluated by comparing our results with the EZ defined by a clinician

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Results

PRE-SURGERY EZ

	1-30Hz		30-80 Hz		1-80 Hz	
	TP (%)	FP (%)	TP (%)	FP (%)	TP (%)	FP (%)
Pt1	100	86	90	58	100	59
Pt2	100	51	44	58	60	50
Pt3	67	58	78	69	89	48
Pt4	50	60	58	63	75	62
Pt5	100	47	100	46	100	50
Pt6	86	55	86	32	86	53
Pt7	82	47	76	56	76	50
Pt8	100	69	100	50	100	64
Pt9	89	55	78	65	100	62
Pt10	83	57	67	46	75	57
Pt11	92	58	75	56	92	56
Pt12	100	58	56	55	89	61
Pt13	67	54	76	35	71	47
Pt14	59	51	59	64	71	55
Mean	**82,6923**	**55,3846**	**73,3077**	**53,4615**	83,3846	55
St. Dev.	**16,98**	**5,85**	**16,54**	**11,32**	12,98	5,86

TP=True positive, % of correctly classified leads.

FP=false positive, % of leads classified as belonging to EZ, but not included in the EZ identified by clinician and/or not in the region removed by surgery.

RESECTED EZ

	1-30Hz		30-80 Hz		1-80 Hz	
	TP (%)	FP (%)	TP (%)	FP (%)	TP (%)	FP (%)
Pt1	100	86	100	60	100	60
Pt2	100	54	60	56	100	48
Pt3	67	58	100	67	100	49
Pt4	71	58	71	62	86	62
Pt5	100	47	100	46	100	50
Pt6	100	55	100	38	100	53
Pt7	100	53	100	58	100	53
Pt8	100	69	100	50	100	64
Pt9	89	55	78	65	100	62
Pt10	100	58	100	45	100	57
Pt11	100	59	75	57	100	57
Pt12	100	58	56	55	89	61
Pt13	85	52	92	37	85	48
Pt14	100	49	100	60	100	55
Mean	**93,7143**	**57,9286**	**88**	**54**	97,1429	55,6429
St. Dev.	**11,51**	**9,62**	**16,49**	**9,45**	5,74	5,61

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Comments

- Most (all?) of the studies are retrospective studies including only patients with good outcome
- Most are based on analysis of seizures or transition to seizures epochs
- Non consensus about the protocols
- Small sample size
- Different type of epilepsy
- Different Algorithms
- Mostly group studies in which inferences are made about a population BUT Neurosurgical planning requires single-subject–specific information, spatial precision, maximizing sensitivity

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Comments

Key point: Changes in EZ connectivity can be extracted from SEEG signals analyzing short segments of interictal activity
(also Bettus et al., 2008; Wilke et al., 2011)

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Comments: What Next?

- The appropriateness of this approach in correctly identify the EZ should be validated on large group of patients, including also patients not in Engel class I
- Much work is needed to understand which measures of connectivity and topology (and protocol), work best to delineate the EZ region
- At present visual analysis of SEEG signals by clinical experts remains the "gold standard", but it can be complemented by quantitative automatic methods, which offer more objective criteria for EZ assessment

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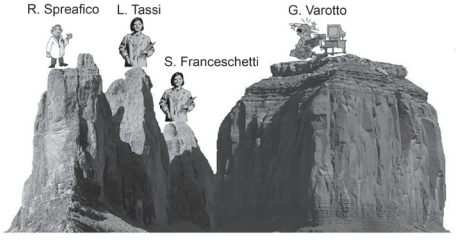
Thanks



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Graph analysis of epileptogenic networks in human partial epilepsy

*†Christopher Wilke, ‡Gregory Worrell, and *†Bin He

*Department of Biomedical Engineering, University of Minnesota, Minneapolis, Minnesota, U.S.A.; †Center for Neuroengineering, University of Minnesota, Minneapolis, Minnesota, U.S.A.; and ‡Department of Neurology, Mayo Clinic, Rochester, Minnesota, U.S.A.

SUMMARY

Purpose: The current gold standard for the localization of the cortical regions responsible for the initiation and propagation of the ictal activity is through the use of invasive electrocorticography (ECoG). This method is utilized to guide surgical intervention in cases of medically intractable epilepsy by identifying the location and extent of the epileptogenic focus. Recent studies have proposed mechanisms in which the activity of epileptogenic cortical networks, rather than discrete focal sources, contributes to the generation of the ictal state. If true, selective modulation of key network components could be employed for the prevention and termination of the ictal state.

Methods: Here, we have applied graph theory methods as a means to identify critical network nodes in cortical networks during both ictal and interictal states. ECoG recordings were obtained from a cohort of 25 patients undergoing presurgical monitoring for the treatment of intractable epilepsy at the Mayo Clinic (Rochester, MN, U.S.A.).

Key Findings: One graph measure, the betweenness centrality, was found to correlate with the location of the resected cortical regions in patients who were seizure-free following surgical intervention. Furthermore, these network interactions were also observed during random nonictal periods as well as during interictal spike activity. These network characteristics were found to be frequency dependent, with high frequency gamma band activity most closely correlated with improved postsurgical outcome as has been reported in previous literature.

Significance: These findings could lead to improved understanding of epileptogenesis. In addition, this theoretically allows for more targeted therapeutic interventions through the selected modulation or disruption of these epileptogenic networks.

KEY WORDS: Seizure, Source localization, Graph analysis, Electrocorticography.

Epilepsia, 52(1):84–93, 2011

Sistema Sanitario Regione Lombardia

Ictal-onset localization through connectivity analysis of intracranial EEG signals in patients with refractory epilepsy

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*Medical Image and Signal Processing Group, Department of Electronics and Information Systems, Ghent University – iMinds, Ghent, Belgium; †Laboratory for Clinical and Experimental Neurophysiology, Neurobiology and Neuropsychology, Department of Neurology, Ghent University Hospital, Ghent, Belgium; ‡Electronic Circuit Design and Realization, Department of Industrial Sciences and Technology, College University of Bruges-Ostend, Ostend, Belgium; §Department of Neurosurgery, Ghent University Hospital, Ghent, Belgium; and ¶Molecular Imaging Center Antwerp, Faculty of Medicine, Antwerp University, Antwerp, Belgium

SUMMARY

Purpose: Fifteen percent to 25% of patients with refractory epilepsy require invasive video–electroencephalography (iEEG) monitoring (IVEM) to precisely delineate the ictal-onset zone. This delineation based on the recorded intracranial EEG (iEEG) signals occurs visually by the epileptologist and is therefore prone to human mistakes. The purpose of this study is to investigate whether effective connectivity analysis of intracranially recorded EEG during seizures provides an objective method to localize the ictal-onset zone.

Methods: In this study data were analyzed from eight patients who underwent IVEM at Ghent University Hospital in Belgium. All patients had a focal ictal onset and were seizure-free following resective surgery. The effective connectivity pattern was calculated during the first 20 s of ictal rhythmic iEEG activity. The out-degree, which is reflective of the number of outgoing connections, was calculated for each electrode contact for every single seizure during these 20 s. The seizure specific out-degrees were summed per patient to obtain the total out-degree. The electrode contact with the highest total out-degree was considered indicative of localization of the ictal-onset zone. This result was compared to the conclusion of the visual analysis of the epileptologist and the resected brain region segmented from postoperative magnetic resonance imaging (MRI).

Key findings: In all eight patients the electrode contact with the highest total out-degree was among the contacts identified by the epileptologist as the ictal onset. This contact, that we named "the driver," always laid within the resected brain region. Furthermore, the patient-specific connectivity patterns were consistent over the majority of seizures.

Significance: In this study we demonstrated the feasibility of correctly localizing the ictal-onset zone from iEEG recordings by using effective connectivity analysis during the first 20 s of ictal rhythmic iEEG activity.

KEY WORDS: Ictal-onset zone localization, Intracranial EEG, Connectivity.

Epilepsia, 54(8):1409–1418, 2013

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Graph Measures of Node Strength for Characterizing Preictal Synchrony in Partial Epilepsy

Sandra Courtens,[1] Bruno Colombet,[1] Agnès Trébuchon,[1,2] Andrea Brovelli,[3] Fabrice Bartolomei,[1,2,4] and Christian G. Bénar[1,*]

Abstract

The reference electrophysiological pattern at seizure onset is the ''rapid discharge,'' as visible on intracerebral electroencephalography (EEG). This discharge typically corresponds to a decrease of synchrony across brain areas. In contrast, the preictal period can exhibit patterns of increased synchrony, which can be quantified by network measures. Our objective was to compare preictal synchrony with a quantification of the rapid discharge as provided by the epileptogenicity index (EI). We investigated 24 seizures from 12 patients recorded by stereotaxic EEG (SEEG). Seizures were classified visually as containing preictal synchrony or not. We computed pairwise nonlinear correlation (h^2) across channels in the 8 sec preceding the rapid discharge. The sum of ingoing and outgoing links (IN and OUT node strength), as well as the sum of all links (total strength, TOT) were computed for each region. We tested several filtering schemes, and quantified the capacity of each strength measure to serve as a detector of regions with high EI values using a receiver operating characteristic (ROC) analysis. We found that the best correspondence between node strength and EI was obtained for the OUT and TOT measures, for signals filtered in the 15–40 Hz band—that is, for the band corresponding to the spiky part of epileptic discharges. In agreement with these results, we also found that the ROC results were improved when considering only seizures with visible synchronous patterns in the preictal period. Our results suggest that measuring strength of preictal connectivity graphs can bring useful clinical information on the epileptogenic zone.

BRAIN CONNECTIVITY
Volume 6, Number 7, 2016

Sistema Sanitario Regione Lombardia

Localization of epileptogenic zone based on graph analysis of stereo-EEG

Yong-Hua Li[a], Xiao-Lai Ye[b], Qiang-Qiang Liu[b], Jun-Wei Mao[a], Pei-Ji Liang[a], Ji-Wen Xu[b,*], Pu-Ming Zhang[a,*]

[a] School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China
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A B S T R A C T

Localization of the epileptogenic zone (EZ) is essential for the successful surgical treatment of medically intractable epilepsy. In the present study, stereo-EEG (SEEG) recordings were obtained from seven patients underwent presurgical evaluation for treatment of intractable epilepsy. Partial directed coherence (PDC) analysis was applied to construct peri-ictal effective connectivity networks. The graphic measures, in-degree, out-degree and betweenness centrality, were evaluated to localize the EZ. A receiver operating characteristic (ROC) analysis was used to quantify the localization accuracy. We found that the in-degree coincided well with the EZ identified by epileptologists' visual inspection in all seven patients who had a significant improvement in seizure outcomes, however, the other two measures were effective only in some cases. Furthermore, in all seven patients the electrode contact with the highest in-degree was always located within the EZ identified by epileptologists' visual inspection. These results indicate that the graph theory is an effective method to localize the EZ when suitable graphic measures were chosen. Furthermore, the in-degree was the most effective measure among the three graphic measures in localizing the EZ when the PDC method was used.

Epilepsy Research 128 (2016) 149–157

Sistema Sanitario Regione Lombardia

Dynamic Network Connectivity Analysis to Identify Epileptogenic Zones Based on Stereo-Electroencephalography

Jun-Wei Mao[1], Xiao-Lai Ye[2], Yong-Hua Li[1], Pei-Ji Liang[1], Ji-Wen Xu[2] and *Pu-Ming Zhang[1]**

Objectives: Accurate localization of epileptogenic zones (EZs) is essential for successful surgical treatment of refractory focal epilepsy. The aim of the present study is to investigate whether a dynamic network connectivity analysis based on stereo-electroencephalography (SEEG) signals is effective in localizing EZs.

Methods: SEEG data were recorded from seven patients who underwent presurgical evaluation for the treatment of refractory focal epilepsy and for whom the subsequent resective surgery gave a good outcome. A time-variant multivariate autoregressive model was constructed using a Kalman filter, and the time-variant partial directed coherence was computed. This was then used to construct a dynamic directed network model of the epileptic brain. Three graph measures (in-degree, out-degree, and betweenness centrality) were used to analyze the characteristics of the dynamic network and to find the important nodes in it.

Results: In all seven patients, the indicative EZs localized by the in-degree and the betweenness centrality were highly consistent with the clinically diagnosed EZs. However, the out-degree did not indicate any significant differences between nodes in the network.

Conclusions: In this work, a method based on ictal SEEG signals and effective connectivity analysis localized EZs accurately. The results suggest that the in-degree and betweenness centrality may be better network characteristics to localize EZs than the out-degree.

Frontiers in Computational Neuroscience | www.frontiersin.org October 2016 | Volume 10 | Article 11:

Sistema Sanitario Regione Lombardia

SozRank: A new approach for localizing the epileptic seizure onset zone

Yonathan Murin[1], Jeremy Kim[1], Josef Parvizi[2], Andrea Goldsmith[1]*

Abstract

Epilepsy is one of the most common neurological disorders affecting about 1% of the world population. For patients with focal seizures that cannot be treated with antiepileptic drugs, the common treatment is a surgical procedure for removal of the seizure onset zone (SOZ). In this work we introduce an algorithm for automatic localization of the seizure onset zone (SOZ) in epileptic patients based on electrocorticography (ECoG) recordings. The proposed algorithm builds upon the hypothesis that the abnormal excessive (or synchronous) neuronal activity in the brain leading to seizures *starts in the SOZ* and then *spreads* to other areas in the brain. Thus, when this abnormal activity starts, signals recorded at electrodes close to the SOZ should have a relatively large *causal influence* on the rest of the recorded signals. The SOZ localization is executed in two steps. First, the algorithm represents the set of electrodes using a directed graph in which nodes correspond to recording electrodes and the edges' weights quantify the *pair-wise* causal influence between the recorded signals. Then, the algorithm infers the SOZ from the estimated graph using a variant of the *PageRank* algorithm followed by a novel post-processing phase. Inference results for 19 patients show a close match between the SOZ inferred by the proposed approach and the SOZ estimated by expert neurologists (success rate of 17 out of 19).

PLOS Computational Biology | https://doi.org/10.1371/journal.pcbi.1005953 January 30, 2018

Sistema Sanitario Regione Lombardia

Using network analysis to localize the epileptogenic zone from invasive EEG recordings in intractable focal epilepsy

Adam Li[1], Bhaskar Chennuri[2], Sandya Subramanian[1], Robert Yaffe[3], Steve Gliske[2], William Stacey[2], Robert Norton[4], Austin Jordan[1], Kareem A. Zaghloul[5], Sara K. Inati[5], Shubhi Agrawal[1], Jennifer J. Haagensen[1], Jennifer Hopp[1], Chalita Atallah[5], Emily Johnson[2], Nathan Crone[3], William S. Anderson[6], Zach Fitzgerald[6], Juan Bulacio[6], John T. Gale[6], Sridevi V. Sarma[1], and Jorge Gonzalez-Martinez[6]

ABSTRACT

Treatment of medically intractable focal epilepsy (MIFE) by surgical resection of the epileptogenic zone (EZ) is often effective provided the EZ can be reliably identified. Even with the use of invasive recordings, the clinical differentiation between the EZ and normal brain areas can be quite challenging, mainly in patients without MRI detectable lesions. Consequently, despite relatively large brain regions being removed, surgical success rates barely reach 60–65%. Such variable and unfavorable outcomes associated with high morbidity rates are often caused by imprecise and/or inaccurate EZ localization. We developed a localization algorithm that uses network-based data analytics to process invasive EEG recordings. This network algorithm analyzes the centrality signatures of every contact electrode within the recording network and characterizes contacts into susceptible EZ based on the centrality trends over time. The algorithm was tested in a retrospective study that included 42 patients from four epilepsy centers. Our algorithm had higher agreement with EZ regions identified by clinicians for patients with successful surgical outcomes and less agreement for patients with failed outcomes. These findings suggest that network analytics and a network systems perspective of epilepsy may be useful in assisting clinicians in more accurately localizing the EZ.

Network Neuroscience **2(2), 218–240.**

Sistema Sanitario Regione Lombardia

Identification of the epileptogenic zone of temporal lobe epilepsy from stereo-electroencephalography signals: A phase transfer entropy and graph theory approach

Meng-yang Wang[a], Jing Wang[a], Jian Zhou[b], Yu-guang Guan[b], Feng Zhai[b], Chang-qing Liu[b], Fei-fei Xu[a], Yi-xian Han[a], Zhao-fen Yan[a], Guo-ming Luan[b,*]

A B S T R A C T

The aim of this research is to apply an approach based on phase transfer entropy (PTE) and graph theory to study the interactions between the stereo-electroencephalography (SEEG) activities recorded in multilobar origin, in order to evaluate their ability to detect the epileptogenic zone (EZ) of temporal lobe epilepsies (TLE). Forty-three patients were included in this retrospective study. Five to sixteen (median = 12) multilead electrodes were implanted per patient, and, for each patient, a sub-set of between 10 and 32 (median = 22) bipolar derivations was selected for analysis. The leads were classified into the onset leads (OLs), the early propagation leads (EPLs), and the rest of the leads (RLs). The results showed that a significantly different dynamic trend of the out/in ratio (more obvious in the gamma band) distinguishes the OLs from RLs in the 23 patients who were seizure-free not only during the ictal event (significant elevation), but also during the inter-,pre-, late-ictal periods, and especially in the post-ictal (sharp decline) state. However, in the 20 patients who were not-seizure-free, the differences between the OLs and RLs during the post-ictal period were not found in any frequency band. The dynamic trend was used to predict surgical outcome, and the results showed that the sensitivity was 91% and the specificity was 70%. In brief, this study indicates that our approach may add new and valuable information, providing efficient quantitative measures useful for localizing the EZ.

NeuroImage: Clinical 16 (2017) 184–195

Sistema Sanitario Regione Lombardia

Betweenness centrality of intracranial electroencephalography networks and surgical epilepsy outcome

Bartosz T. Grobelny [a], Dennis London [a], Travis C. Hill [a], Emily North [a], Patricia Dugan [b], Werner K. Doyle [a,c]

Objective: We sought to determine whether the presence or surgical removal of certain nodes in a connectivity network constructed from intracranial electroencephalography recordings determines postoperative seizure freedom in surgical epilepsy patients.
Methods: We analyzed connectivity networks constructed from peri-ictal intracranial electroencephalography of surgical epilepsy patients before a tailored resection. Thirty-six patients and 123 seizures were analyzed. Their Engel class postsurgical seizure outcome was determined at least one year after surgery. Betweenness centrality, a measure of a node's importance as a hub in the network, was used to compare nodes.
Results: The presence of larger quantities of high-betweenness nodes in interictal and postictal networks was associated with failure to achieve seizure freedom from the surgery ($p < 0.001$), as was resection of high-betweenness nodes in three successive frequency groups in mid-seizure networks ($p < 0.001$).
Conclusions: Betweenness centrality is a biomarker for postsurgical seizure outcomes. The presence of high-betweenness nodes in interictal and postictal networks can predict patient outcome independent of resection. Additionally, since their resection is associated with worse seizure outcomes, the mid-seizure network high-betweenness centrality nodes may represent hubs in self-regulatory networks that inhibit or help terminate seizures.
Significance: This is the first study to identify network nodes that are possibly protective in epilepsy.

Clinical Neurophysiology 129 (2018) 1804–1812

Sistema Sanitario Regione Lombardia

CRITICAL REVIEW AND INVITED COMMENTARY

Defining epileptogenic networks: Contribution of SEEG and signal analysis

*†Fabrice Bartolomei, *†Stanislas Lagarde, ‡Fabrice Wendling, *†Aileen McGonigal, *Viktor Jirsa, §Maxime Guye, and *Christian Bénar

Epilepsia, 58(7):1131–1147, 2017
doi: 10.1111/epi.13791

SUMMARY

Epileptogenic networks are defined by the brain regions involved in the production and propagation of epileptic activities. In this review we describe the historical, methodologic, and conceptual bases of this model in the analysis of electrophysiologic intracerebral recordings. In the context of epilepsy surgery, the determination of cerebral regions producing seizures (i.e., the "epileptogenic zone") is a crucial objective. In contrast with a traditional focal vision of focal drug-resistant epilepsies, the concept of epileptogenic networks has been progressively introduced as a model better able to describe the complexity of seizure dynamics and realistically describe the distribution of epileptogenic anomalies in the brain. The concept of epileptogenic networks is historically linked to the development of the stereoelectroencephalography (SEEG) method and subsequent introduction of means of quantifying the recorded signals. Seizures, and preictal and interictal discharges produce clear patterns on SEEG. These patterns can be analyzed utilizing signal analysis methods that quantify high-frequency oscillations or changes in functional connectivity. Dramatic changes in SEEG brain connectivity can be described during seizure genesis and propagation within cortical and subcortical regions, associated with the production of different patterns of seizure semiology. The interictal state is characterized by networks generating abnormal activities (interictal spikes) and also by modified functional properties. The introduction of novel approaches to large-scale modeling of these networks offers new methods in the goal of better predicting the effects of epilepsy surgery. The epileptogenic network concept is a key factor in identifying the anatomic distribution of the epileptogenic process, which is particularly important in the context of epilepsy surgery.

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Isabella D'Andrea Meira (Brazil)

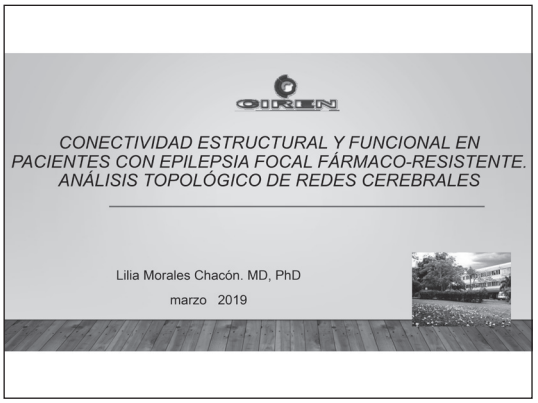
UNDERSTANDING THE VAGUS AFFERENT NETWORK AND ITS ROLE IN TRANSLATIONAL CONNECTOMICS

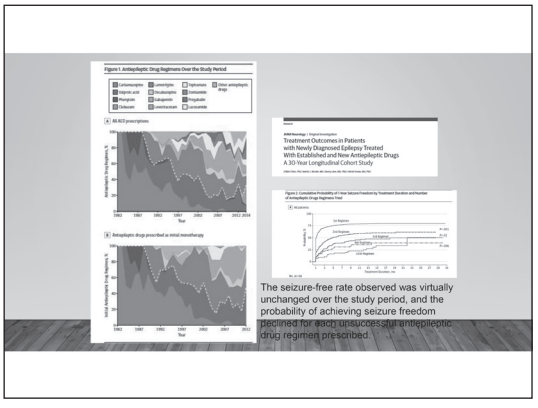
Matthias Koepp (England)

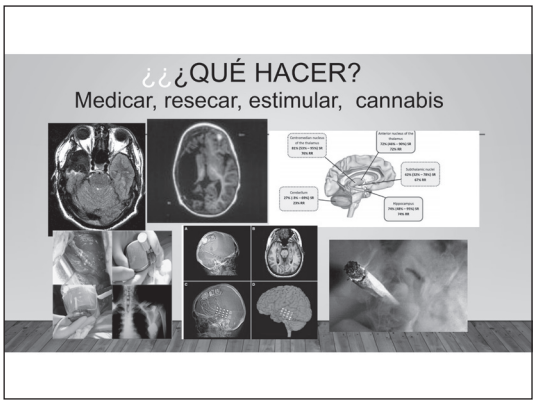
EFFECT OF PHARMACOLOGICAL AND SURGICAL INTERVENTIONS ON COGNITIVE NETWORKS

Lilia Morales (Cuba)

CONNECTIVITY DERIVED FROM EEG AND NEUROIMAGING IN FOCAL EPILEPSIES



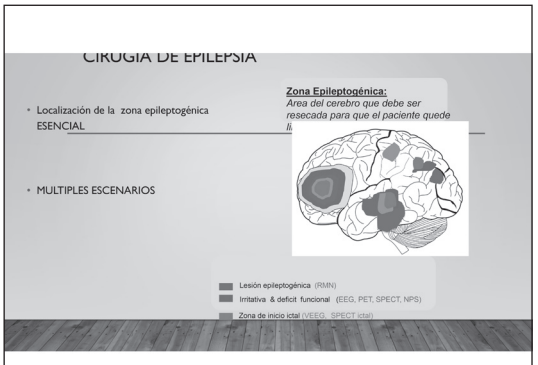




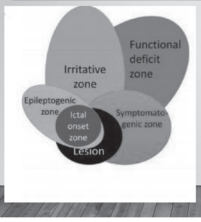
Objetivo de la cirugía epilepsia

Resección completa o desconexión de la " zona epileptogénica " o interrupción de la " red epileptogénica"

Este objetivo debe garantizarse con la preservación de la corteza elocuente.



SOBRELAPAMIENTO VARIABLE DE LA ZE CON LAS PRINCIPALES ÁREAS CORTICALES IMPLICACIONES



No prueba equivalente a ZE

No existe un test que permita estimar la ZE

Definir la zona de inicio ictal no es similar a definir la ZE

¿¿¿QUÉ HACER?

Very High-Frequency Oscillations: Novel Biomarkers of the Epileptogenic Zone

Milan Brázdil, MD, PhD,[1,2] Martin Pail, MD, PhD,[1] Josef Halámek, PhD,[3,4] Filip Plešinger, PhD,[3] Jan Cimbalník,[4] Robert Roman, MD, PhD,[1] Petr Klimeš,[3] Pavel Daniel,[1,2] Jan Chrastina, MD, PhD,[1] Eva Brichtová, MD, PhD,[1] Ivan Rektor, MD, PhD,[1,2] Gregory A. Worrell, MD, PhD,[5] and Pavel Jurák, PhD[3,4]

Automated Online Quantification Method for ^{18}F-FDG Positron Emission Tomography/CT Improves Detection of the Epileptogenic Zone in Patients with Pharmacoresistant Epilepsy

Vanessa Cristina Mendes Coelho[1], Marcia E. Morita[1], Barbara J. Amorim[1], Celso Dario Ramos[2], Clarissa L. Yasuda[1], Helder Tedeschi[1], Enrico Ghizoni[2] and Fernando Cendes[1]

Clinical EEG and Neuroscience
1–10
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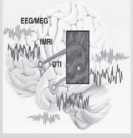
DOI: 10.1177/1550059414522231
eeg.sagepub.com
SAGE

Improving Electroencephalographic Source Localization of Epileptogenic Zones With Time-Frequency Analysis

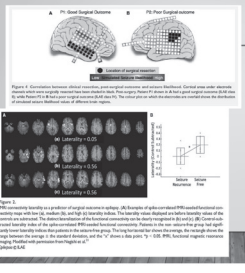
Imaging the seizure onset zone with stereo-electroencephalography

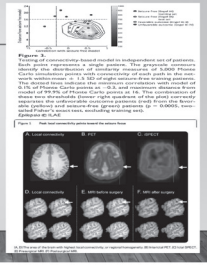
Olivier David,[1,2,3] Thomas Blauwblomme,[4] Anne-Sophie Job,[5] Stéphan Chabardès,[1,2,6] Dominique Hoffmann,[4] Lorella Minotti[1,2] and Philippe Kahane[1,2,7]

- Las técnicas de neuroimágenes se utilizan actualmente para investigar la integración de regiones cerebrales especializadas funcionalmente



¿CONECTIVIDAD?





Las técnicas de mapeo cerebrales (como la DTI) producen patrones de conectividad más precisos, permitiendo un enfoque nuevo y multidisciplinario que permite estudiar sistemas complejos.

Rubinov M, Sporns O. Complex network measures of brain connectivity: Uses and interpretations. Neuroimage. 2010; 52(3), 1059-1069.

Table 1. Common modalities used to study functional connectivity in focal epilepsy

Modality	Advantages	Disadvantages
MEG	Noninvasive; whole-brain coverage; high spatial resolution; high temporal resolution	Source reconstruction challenging from deep structures; total recordings very challenging; not widely available
fMRI	Noninvasive; whole-brain coverage; reliable signals from deep structures; relatively high spatial resolution; widely available	Low temporal resolution; indirect measurement of neuronal activity; total recordings very challenging
ECoG	High spatial and temporal resolution; more direct measurement of neuronal activity; reliable total recordings	Highly invasive; limited to brain areas covered with electrodes; no data from normal controls
Scalp EEG	Noninvasive; high temporal resolution; widely available and inexpensive	Low spatial resolution; signal degradation by skull and scalp

ECoG electrocorticography; EEG electroencephalography; fMRI functional magnetic resonance imaging; MEG magnetoencephalography.

Englot D, Konrad PE, Morgan VL. Regional and global connectivity disturbances in focal epilepsy, related neurocognitive sequelae, and potential mechanistic underpinnings. Epilepsia. 2016;57(10):1546–1557.

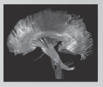
Con esta técnica no es posible determinar la direccionalidad de las fibras, además de que es imposible reconstruir de manera totalmente detallada los caminos neuronales, especialmente cuando las fibras se ramifican o se cruzan.

Cuenca I. Caracterización de la conectividad estructural cerebral basada en la teoría de redes complejas (tesis doctoral). Valencia: Escuel Técnica Superior de Ingenieros Industriales; 2017.

Conceptos básicos

- Friston: define el término conectividad como la correlación temporal entre los datos de la activación de dos áreas cerebrales. Esta medida indica una sincronización sistemática entre áreas, modulada por diversas variables. La sincronización se considera como evidencia de conectividad funcional.

Conectividad estructural se refiere a las conexiones físicas o estructurales (sinápticas) que unen grupos de neuronas, y a sus propiedades biofísicas estructurales asociadas, como fuerza o efectividad sináptica.



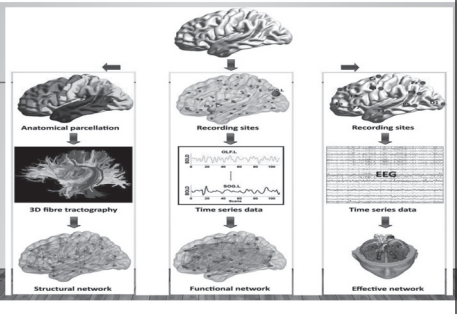
MEDIDAS DE CONECTIVIDAD ANATOMICA

medidas de conectividad entre regiones: Fuerza de Conexión Anatómica (FCA),Densidad de Conexión Anatómica (DCA) y Probabilidad de Conexión Anatómica (PCA).

FCA provee una estimación del flujo potencial de información entre cualquier par de regiones, considerando que dicho flujo es proporcional a la cantidad de fibras nerviosas compartidas por estas.

DCA es una medida de la fracción del área externa de las regiones que se encuentra conectada conrespecto al área externa total de ambas, es, por tanto, una medida que intenta corregir por el tamaño de las regiones involucradas en cada conexión

ACP es una medida de la probabilidad de conexión, al menos por una fibra nerviosa, entre estas regiones. Dicha medida permite inferir si dos regiones se pueden encontrar vinculadas funcionalmente de forma directa, sin tener en cuenta las características de la conexión
.



Conceptos básicos

Conectividad funcional se refiere al grado con el cual la actividad en un área se correlaciona con la actividad de otra área, o la sincronización temporal de activación de dos áreas cerebrales.

Describe el conjunto de efectos causales de un sistema neuronal sobre otro.

Análisis topológico de redes cerebrales

Si tenemos las redes que representan las conexiones anatómicas cerebrales, ¿qué hacer con ellas?, ¿cómo analizar la información que contienen?, ¿qué conclusiones biológicas pueden extraerse?

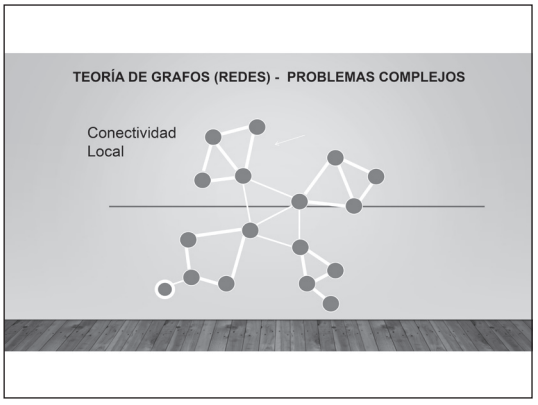
Medidas topológicas, propias de la teoría de grafos, que buscan caracterizar las propiedades de la red en su forma general (como eficiencia global, índice de "mundo-pequeño", longitud del camino medio, configuración de motivos estructurales) y las de sus nodos locales (eficiencia local, centralidad, vulnerabilidad), brindando valores cuantitativos que reflejan las facultades intrínsecas para apoyar o soportar el flujo potencial de actividad neural.

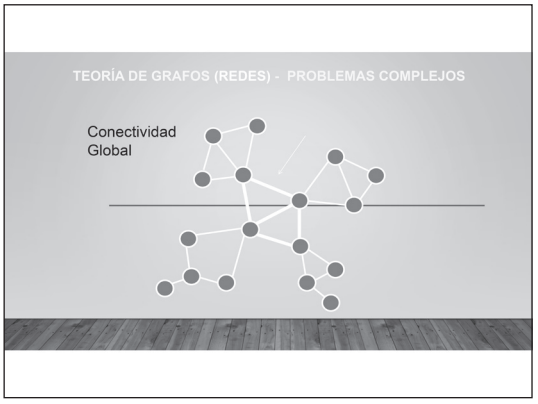
Conceptos básicos

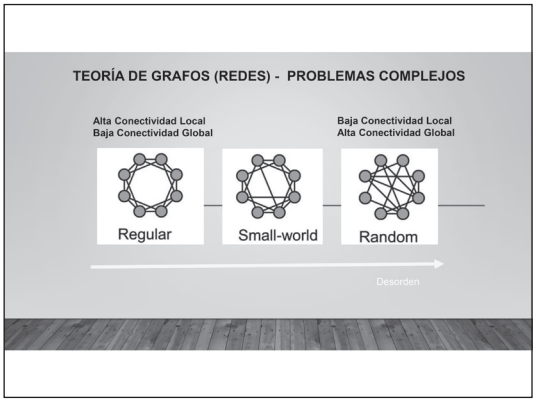
Teoría de grafos: estudia las teorías de los gráficos en conjunto no vacío utilizando términos como: vértices o nodos y selección de pares de vértices, las aristas orientados o no.

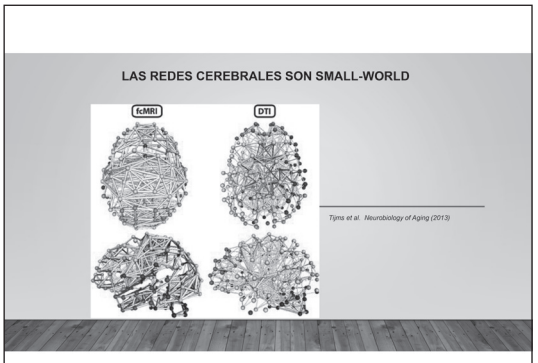
Diagrama de un grafo con 6 vértices y 7 aristas.

TEORÍA DE GRAFOS (REDES) - PROBLEMAS COMPLEJOS


TEORÍA DE GRAFOS (REDES) - PROBLEMAS COMPLEJOS
Conectividad
Local


TEORÍA DE GRAFOS (REDES) - PROBLEMAS COMPLEJOS
Conectividad
Global


TEORÍA DE GRAFOS (REDES) - PROBLEMAS COMPLEJOS
Alta Conectividad Local
Baja Conectividad Global
Baja Conectividad Local
Alta Conectividad Global
Regular
Small-world
Random
Desorden


LAS REDES CEREBRALES SON SMALL-WORLD
fcMRI
DTI
Tijms et al. Neurobiology of Aging (2013)

ANTECEDENTES
Conectividad - EPILEPSIA

- La epilepsia focal (EF) considerada tradicionalmente como un trastorno regional ya en la actualidad s ha demostrado como una alteración de redes neurales.
- Los cambios en la conectividad se relacionan con la duración y severidad de la enfermedad sugiriend una reorganización progresiva de la conectividad.
- A pesar de todos los métodos y evaluaciones multidisciplinarias para estimar la ZE, luego de la cirugí un 20% de los pacientes para la epilepsia del lóbulo temporal (ELT) y un 40% para la epilepsi extratemporal (ExT), recurren con crisis, lo que evidencia la necesidad de nuevos acercamientos par identificar la ZE y con ello lograr una mejor estrategia para el manejo de estos pacientes.
- Existe incremento de la conectividad en la zona epileptogénica (ZE) acompañada de una disminució distal en las redes

APLICACIONES PROMISORIAS
Conectividad - EPILEPSIA

- La con~~ectividad cerebral funcional obtenida a partir de los registros de EEG puede ser utilizada~~ para localizar la ZE (Gersch and Goddard (1970), Gotman (1983), (Mars et al., 1985), Gotman (1987) Lieb et al. (1987), (Bartolomei et al.,1999, 2001, 2004, 2005), Wendling et al., 2003, 2010) Ponten et al. (2007), Bialonski and Lehnertz (2013) y para la predicción de las crisis Morrisseau et al. (2007) and Schulze-Bonhage et al. (2011).
- Los estudios de conectividad en EF pueden dar mejores estrategias para la localización de la ZE, en la predicción de la evolución quirúrgica y una mejor comprensión de las implicaciones neurofisiológicas en la recurrencia de las crisis.
- Otras aplicaciones promisorias del análisis de la conectividad funcional es la investigación de los mecanismos de acción de los tratamientos de neuromodulación, asi como la identificación mas precisa de blancos para cirugía de epilepsia. En este sentido resulta importante avanzar en la comprension de la correlacion entre los cambios en la organizacion de la red funcional producto de la cirugía y la evolución clínica postquirúrgica

LINEAS ACTUALES
Conectividad - EPILEPSIA

- Minimizar las implicaciones de la metodología en los resultados obtenidos de conectividad en las epilepsies focales.
- Determinar cuales alteraciones de la conectividad son reversibles luego de la cirugia de epilepsia.
- Identificar la zona epileptogenica utilizando métodos cuantitativos

Predecir la evolución post quirúrgica.

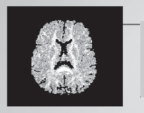
Problema: ¿Podrían las propiedades topológicas de las redes, a partir de la conectividad estructural derivada de la IRM-DTI y funcional del EEG en pacientes con epilepsia focal farmacorresistente (EFFR) del lóbulo temporal y extratemporal, ofrecer una aproximación cuantitativa a la estimación de la ZE?

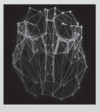
DATOS DEMOGRÁFICOS EN PACIENTES CON EFFR DE LÓBULO TEMPORAL Y EXTRATEMPORAL.

Tipo de EFFR/ Condición	ELT no operados n=6	ExT no operados n=6	(p-valor)
Edad	33,3+-15,7	24,6+-10,8	p=0,26
Sexo/Femenino	5	6	p=0,24
Sexo/Masculino	1	-	p=0,42
Edad de inicio de las crisis	12,3+-11,5	9,8+-5,6	p=0,936
Duración de la enfermedad	21+-15,2	13,1+-14,6	p=0,47
Lateralidad/Izquierda	5	2	p=0,08
Lateralidad/Derecha	1	3	p=0,13
Linea media	-	1	p=0,42
Cantidad de FAE/1	2	1	p=0,38
FAE/2	2	2	p=1
FAE/+3	2	3	p=0,47

Observaciones: EFFR, epilepsia focal farmacorresistente; ELT, epilepsia del lóbulo temporal; ExT, epilepsia extratemporal; FAE, fármacos antiepilépticos.

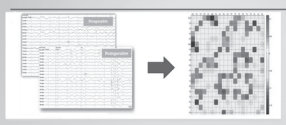
METODOLOGÍA DE REGISTRO Y EVALUACIÓN DE LA CONECTIVIDAD ESTRUCTURAL





- Se realizó en estado de reposo una IRM de 1.5T y DTI de 64 direcciones.
- Con la serie de DTI se estimó una imagen que es el tensor de difusión y a partir de este se obtuvieron diferentes mapas y la función ODF.
- Se realizó un análisis teórico de los datos obtenidos en programación en MatLab 7.0 2014a™
- Se realizó teoría de grafo para describir las propiedades de las redes.

METODOLOGÍA DE REGISTRO Y EVALUACIÓN DE LA CONECTIVIDAD FUNCIONAL



- Se registró en estado funcional de vigilia (duración mínima: 20 minutos).
- Se seleccionaron hasta 15 segmentos libres de artefactos con ojos cerrados.
- Se realizó un análisis teórico (programación en MatLab 10 2014a™).
- Se obtuvieron las matrices de conectividad funcional por banda de frecuencia para todos los pacientes.
- Se realizó teoría de grafo para describir las propiedades de las redes.

PARA LA CUANTIFICACIÓN TOPOLÓGICA DE LA TEORÍA DE GRAFOS

- Índice de clusterización: Medida del grado en que los nodos de una red tienden a agruparse entre ellos.
- Eficiencia local: Medida basada en el número mínimo de enlaces entre cada nodo y sus vecinos, reflejo de cuán eficiente puede ser la comunicación entre los vecinos inmediatos de cada nodo.
- Longitud del camino corto: Número mínimo de enlaces que habría que recorrer para ir de un nodo al otro.
- Eficiencia global: Es la inversa del promedio del número mínimo de enlaces entre cada nodo y sus vecinos, en toda la red.

PROPIEDADES TOPOLÓGICAS DE LA RED DERIVADAS DE LAS MATRICES DE CONECTIVIDAD ESTRUCTURAL EN PACIENTES CON EFFR DEL LÓBULO TEMPORAL Y EXTRATEMPORAL.

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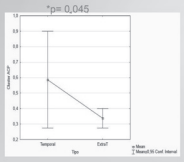
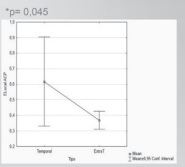




Conocimiento del índice de clusterización y la eficiencia local en la región 5 para la probabilidad de la conectividad anatómica (ACP) en pacientes con epilepsia focal farmacorresistente (EFFR) del lóbulo temporal (ELT) y extratemporal (ExT). (ACP: Eficiencia local; ELT: Lóbulo temporal; ExT: Extratemporal; Tipo: Tipo de EFFR; Mean: Mediana; 95.0% Conf. Interval: IC 95%; *p: Mann–Whitney test.

En pacientes con ELT se encontró un mayor índice de clusterización y eficiencia local para la ACP en la región del hipocampo comparados con ExT.

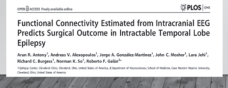

Neuroimaging
RESEARCH PAPER
Medial temporal lobe epilepsy is associated with neuronal fibre loss and paradoxical increase in structural connectivity of limbic structures
Leonardo Bonilha,[1] Erwin Nesland,[1] Gabriel Li Mazzi,[1] Jane E. Joseph,[1] Maria V. Spampinato,[2] Jonathan C Edwards,[1] Ali Tabesh[2]


FULL-LENGTH ORIGINAL RESEARCH
Hippocampal-thalamic wiring in medial temporal lobe epilepsy: Enhanced connectivity per hippocampal voxel
**Vera Drobinskaya, †‡Romain Valabregue, *†Lionel Thivard, †‡Stéphane Lehéricy, **Michel Baulac, ‡Bénédicte Batrancourt, and **Sophie Dupont
Epilepsia, 59(9):1217–1226, 2017
doi: 10.1111/epi.13905


FULL-LENGTH ORIGINAL RESEARCH
Rates and predictors of success and failure in repeat epilepsy surgery: A meta-analysis and systematic review
*Max O. Krucoff ⓘ, †Alvin Y. Chan, *Stephen C. Harward, *Sherwin Rahimpour, ‡John D. Rolston ⓘ, *Carrie Muh, and ‡Dario J. Englot
Epilepsia, **(*):1–10, 2017
doi: 10.1111/epi.13905

PROPIEDADES TOPOLÓGICAS DE LA RED DERIVADAS DE LAS MATRICES DE CONECTIVIDAD FUNCIONAL EN PACIENTES CON EFFR DEL LÓBULO TEMPORAL Y EXTRATEMPORAL.

- No encontramos diferencias significativas en las variables estudiadas para la teoría de grafos, índice de clusterización, eficiencia local, longitud del camino y eficiencia global, a partir de la prueba no paramétrica U Mann–Whitney.


OPEN ACCESS Freely available online
PLOS | ONE
Functional Connectivity Estimated from Intracranial EEG Predicts Surgical Outcome in Intractable Temporal Lobe Epilepsy
Anan S. Antony,[1] Andrew V. Alexopoulos,[2] Jorge A. González-Martínez,[3] John C. Mosher,[1] Lara Jehi,[2] Richard C. Burgess,[2] Norman H. Su[1], Roberto F. Galán[1]*


Seizure
Contents lists available at ScienceDirect
Seizure
Journal homepage: www.elsevier.com/locate/seizure
Neuromagnetic coherence of epileptic activity: An MEG study
Ying Wu[a,b,*], Zheng Ge[a,*], Rui Zhang[c], Hongxi Liu[c], Qiqi Chen[c], Huirui Zhao[c], Yan Yin[c], Shuxin Li[a], Hanzi Jiang[a]

RELACIÓN ENTRE LAS PROPIEDADES TOPOLÓGICAS DE LA RED A PARTIR DE LA CONECTIVIDAD ESTRUCTURAL Y FUNCIONAL

Para abordar este objetivo se comprobó las diferencias entre las variables de las propiedades topológicas de la red derivadas de la IRM-DTI y el EEG, mediante un t test, donde todas las variables resultaron diferentes estadísticamente significativas.

Relación entre la conectividad funcional y estructural para la eficiencia local, en pacientes con ELT, por regiones correspondientes a la ZPE, Correlación de Spearman.

Variables	ELocal ACD Región 5	ELocal ACD Región 6	ELocal ACD Región 7	ELocal ACP Región 2	ELocal ACP Región 3	ELocal ACP Región 4	ELocal ACP Región 5	ELocal ACP Región 6	ELocal ACP Región 7	ELocal ACP Región 8
ELocal Delta	-0,028571	-0,028571	-0,028571	-0,085714	0,028571	-0,028571	-0,028571	-0,028571	-0,028571	-0,028571
ELocal Theta	-0,314286	-0,314286	-0,314286	-0,371429	-0,257143	-0,314286	-0,314286	-0,314286	-0,314286	-0,314286
ELocal Alfa	-0,028571	-0,028571	-0,028571	-0,942857	-0,885714	-0,028571	-0,028571	-0,028571	-0,028571	-0,028571
ELocal Beta	-0,085714	-0,085714	-0,085714	-0,028571	0,085714	-0,085714	-0,085714	-0,085714	-0,085714	-0,085714

Relación entre la conectividad funcional y estructural para la longitud del camino corto, en pacientes con ELT, Correlación de Spearman.

Variables	LCC ACP
LCC Delta	-0,257143
LCC Theta	-0,600000
LCC Alfa	-0,885714
LCC Beta	-0,600000

Relación entre la conectividad funcional y estructural para el índice de clusterización, en pacientes con ExT, por regiones correspondientes a la ZPE, Correlación de Spearman.

Variables	Cluster ACD Región 5
Cluster Delta	-0,371429
Cluster Theta	-0,628571
Cluster Alfa	[illegible]
Cluster Beta	-0,085714

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- Se muestra una relación entre la segregación e integración de la red funcional derivada del EEG y la estructural en pacientes con EFFR específica para la banda alfa.

SCIENTIFIC REPORTS

OPEN **A small change in neuronal network topology can induce explosive synchronization transition and activity propagation in the entire network**

Received: 21 November 2014
Accepted: 12 January 2015
Published online: 11 April 2015

Wenchuang Chiang[1], Yongjun Tian[2], Mulseh Shamim[3] & Zonghua Liu[1]

Neuroimage: Clinical 2 (2013) 503–513

Contents lists available at ScienceDirect

Neuroimage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

What graph theory actually tells us about resting state interictal MEG epileptic activity

Guiomar Niso[a,b,c], Ana Carrasco[d], Maria Gudín[d], Fernando Maestú[d], Francisco del Pozo[d,e], Ernesto Pereda[a]

CONCLUSIONES……

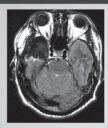
La topología de la red cerebral funcional derivada del EEG en los pacientes con EFFR muestra un comportamiento similar independiente de la localización de la ZE, en tanto, los patrones de conectividad estructural son diferentes. Estos resultados pueden atribuirse a las diferencias en el sustrato neuropatológico y/o la lesión epileptogénica en ambos grupos de pacientes.

La metodología utilizada permite aportar evidencias de las diferencias en las medidas de conectividad derivadas por diferentes modalidades (IRM-DTI y EEG). Se muestra una relación entre la segregación e integración de la red funcional derivada del EEG y la estructural en pacientes con EFFR específica para la banda alfa.

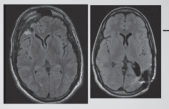
CONCLUSIONES

- Los resultados de este estudio crean las pautas para la cuantificación de la zona potencialmente epileptogenica desde la perspectiva estructura-función, y pueden contribuir a la identificación de biomarcadores cuantitativos para estimar la ZE, y predecir la evolución clínica postquirúrgica.

LAS TECNICAS QUIRURGICAS VARIAN EN DEPENDENCIA DE LA LOCALIZACION Y LA PATOLOGIA



Lobectomias temporales



Lobectomias extratemporales

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MODALIDADES QUIRÚRGICAS RESECTIVAS

Resecciones focales temporales y extratemporales

Table 2. Postoperative seizure outcomes stratified by patient characteristics

	Engel I (%)	Engel II–IV (%)	p
Total	96/0 (67)	47/0 (33)	
(A) Demographics			
Gender			
Female	51 (54)	34 (46)	0.54
Male	44 (57)	41 (46)	
Age at seizure onset, yr (mean ± SEM)	7.1 ± 0.9	6.6 ± 0.9	0.64
n = 96	n = 47		
Age at initial surgery, yr (mean ± SEM)	15.9 ± 1.3	14.9 ± 1.3	0.57
n = 105	n = 50		
Age at last surgery, yr (mean ± SEM)	16.7 ± 1.4	15.7 ± 1.3	0.48
n = 87	n = 77		
(B) Epilepsy characteristics			
Seizure type			
Lateral	95 (61)	62 (39)	<0.01*
Mesiotemporal	145 (41)	205 (59)	
Secondarily generalized seizure			
Yes	23 (58)	34 (63)	<0.08
No	46 (53)	41 (47)	
Predominant seizure type			
FOIA	34 (51)	51 (49)	0.07*
FOIA	38 (45)	46 (55)	
FBTCS	24 (63)	14 (37)	
Duration of epilepsy, yr (mean ± SEM)	13.7 ± 1.4	8.3 ± 1.9	0.18
n = 36	n = 24		
(C) Diagnostics			
MRI findings prior to 1st surgery			
Normal	18 (13)	34 (65)	0.07*
Abnormal	76 (53)	68 (47)	
Electrophysiology			
Congruent	87 (69)	39 (31)	<0.01*
Incongruent	31 (47)	35 (53)	
Invasive monitoring			
Used	47 (53)	41 (48)	<0.01*
Not used	65 (76)	20 (24)	
(D) Surgery/Outcom			
Time between resections, yr (mean ± SEM)	3.1 ± 0.2	3.0 ± 0.3	0.83
n = 96	n = 92		
Resection location, initial surgery			
Temporal	94 (69)	39 (31)	0.04*
Extratemporal	52 (54)	44 (46)	
Resection location, last surgery			
Temporal	119 (64)	112 (40)	<0.01*
Extratemporal	25 (37)	42 (63)	
Side of initial surgery			
Left	47 (48)	50 (52)	0.06
Right	68 (56)	53 (44)	
Side of last surgery			
Left	46 (49)	47 (51)	0.34
Right	65 (56)	51 (44)	
Reason for failure of initial surgery			
Surgical limitations	100 (59)	103 (59)	<0.01*
Disease relapse	3 (13)	48 (68)	

FOIA, focal onset aware (i.e., simple partial); FOIA, focal onset with impaired awareness (i.e., complex partial); MRI, magnetic resonance imaging; SEM, standard error of the mean.
*Statistically significant value (p ≤ 0.05). Data are reported as n values (with corresponding percentage), unless otherwise indicated.

La cirugía de epilepsia ha ofrecido una opción para el control de crisis a largo de plazo en el 60-80% en epilepsia del lóbulo temporal (ELT) y del 40-60% en epilepsia del lóbulo extratemporal (ExT).

Kruoff MO, Chan AY, Harward SC, et al. Rates and predictors of success and failure in repeat epilepsy surgery: A meta-analysis and systematic review. Epilepsia. 2019;60:e20–e25. doi:10.1111/epi.15020

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PATRONES DE CONECTIVIDAD DEL EEG EN FUNCION DE LA EVOLUCION CLINICA POSTQUIRURGICA. UN ESTUDIO EN PACIENTES CON EPILEPSIA DEL LOBULO TEMPORAL FARMACORESISTENTE

SUJETOS Y METODOS
DATOS CLINICOS

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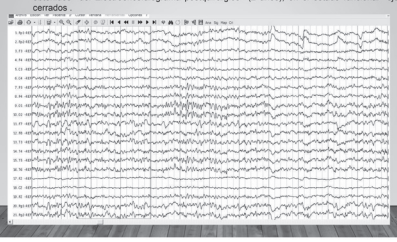
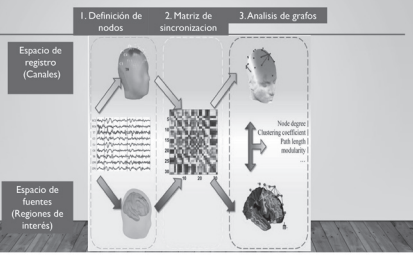
Edad	34.1±7.9 A m:35
Sexo	F: 15 M: 15
Factores de riesgo	Febril convulsion : 25% Encephalitis: 20%
Edad de inicio crisis	12.28±9.3 (8m-29 y) m: 14
No. MAE	2 (1-3)
Tiempo duración crisis	20.21±10.59 (2- 36 A) m: 21
Frecuencia Crisis/m	4-16/m
Lateralización ZE	17 izquierdos 13 derechos
Evolución Clínica Postquirurgica	Izquierdos Engel I 11 Engel II-IV 6 Derechos Engel I 6 Engel II-IV 7

METODOLOGIA

- Se analizó la conectividad funcional calculada a partir de la sincronización entre los electrodos, así como las propiedades de la red derivada de la misma a partir de los datos obtenidos del Electroencefalograma postquirurgico (2 años), en el estado funcional ojos cerrados



EN TODOS LOS SEGMENTOS SELECCIONADOS SE CALCULÓ LA MATRIX DE SINCRONIZACIÓN ESPACIAL (EN INGLÉS SYNCHRONIZATION LIKELIHOOD) . SL ENTRE LOS ELECTRODOS SE DETERMINÓ PARA LAS CUATRO BANDAS DE FRECUENCIA. . EL ANÁLISIS TEÓRICO DE GRAFOS SE REALIZÓ A PARTIR DE LA MATRIX DE SINCRONIZACIÓN DE TODAS LAS COMBINACIONES POSIBLES DE ELECTRODOS.



Cuantificación topológica de la teoria de grafos:

- **Los parámetros evaluados fueron**

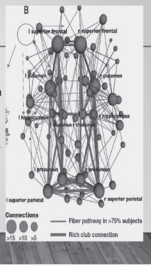
Clustering coefficient (local integration): proporción de conexiones entre los vecinos más cercanos relativo al máximo número de conexiones possibles.

Longitud del camino medio mínimo número de aristas que deben transcurrir de un nodo a otro. Refleja la eficiencia de comunicación en una red.

Eficiencia local refleja cuan conectados están los nodos vecinos (sub-red local)

Eficiencia global refleja cuan conectado está cualquier par de nodos

- **Estos parámetros permiten reflejar la cantidad de información neural que puede ser intercambiada y qué tan optimizadamente pueden realizar estos procesos.**



RESULTADOS

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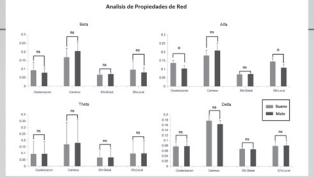
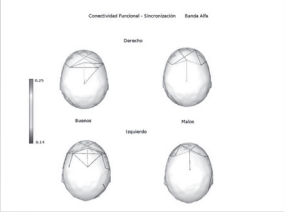


En los pacientes libres de crisis el análisis de las propiedades de la red evidenció para la banda alfa una estructura de red subyacente más organizada con una clusterización significativamente mayor y una mejor eficiencia local que la de los pacientes con persistencia de crisis (t(28)= 2.21, p<0.03, FDR q=0.1; t(28)= 2.22, p<0.03, FDR q=0.1 respectivamente)

RESULTADOS



CIREN
Centro Internacional de Restauración Neurológica

La sincronización espacial dentro de esta banda de frecuencia también mostro un patrón de organización diferente en las regiones anteriores de ambos grupos de pacientes. En el resto de las bandas de frecuencias analizadas no se constataron diferencias en las medidas evaluadas

Se constataron diferencias significativas en la organización de la red en función de la evolución clínica postquirúrgica independientemente de la lateralización de la zona epileptogénica.

CONCLUSIONES

- Las características topológicas de las redes neuronales de los pacientes con ELT sometidos a cirugia que se encuentran libres de crisis se reorganizan de forma más eficiente expresada en los cambios de clusterización y de eficiencia local. Estos cambios topológicos garantizarían un mejor balance en la segregación e integración del procesamiento de información dentro de la red neuronal.
- Estos resultados aunque preliminares sugieren que el resultado postquirúrgico en pacientes con ELT se refleja en la organización funcional de la red.

AGRADECIMIENTOS

YADIRA ROMERO MORALES
KARLA BATISTA
SHEILA BERRILLO
PROYECTO CIRUGIA EPILEPSIA CIREN

Matthias Koepp (England)

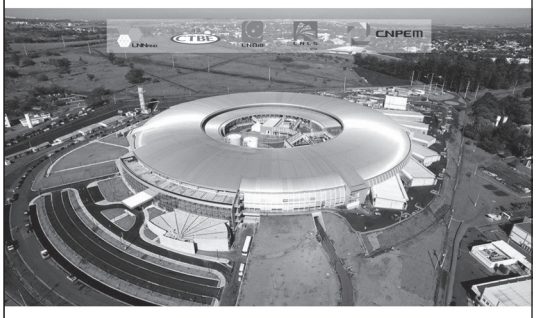
STRUCTURAL AND FUNCTIONAL CONNECTIVITY DURING EPILEPTOGENESIS

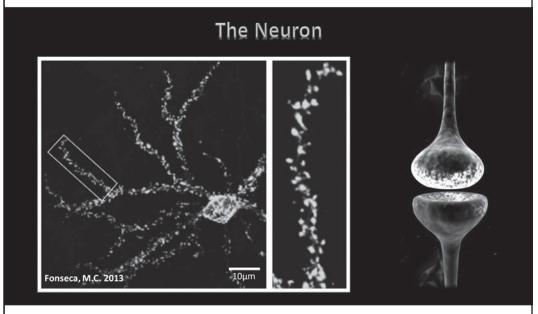
Christophe Bernard (France)

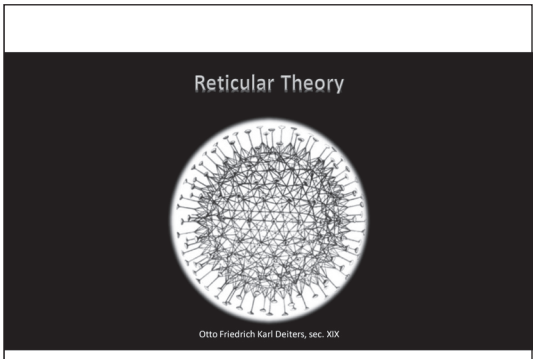
USING STRUCTURAL AND FUNCTIONAL CONNECTOMES TO IMPROVE NEUROSURGERY OUTCOME

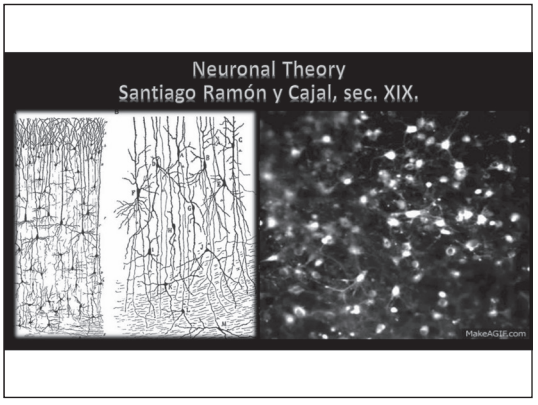
Matheus de Castro Fonseca (Brazil)

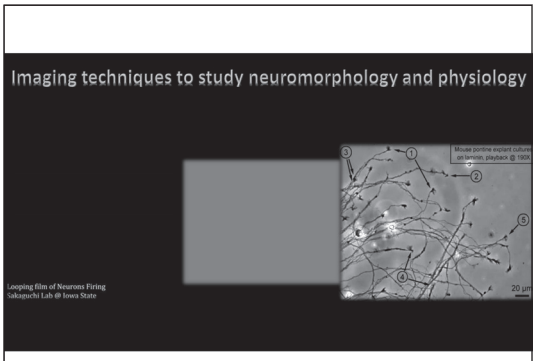
FUTURE TRENDS IN NEURONAL CIRCUITRY IMAGING




The Neuron
Fonseca, M.C. 2013
10μm


Reticular Theory
Otto Friedrich Karl Deiters, sec. XIX

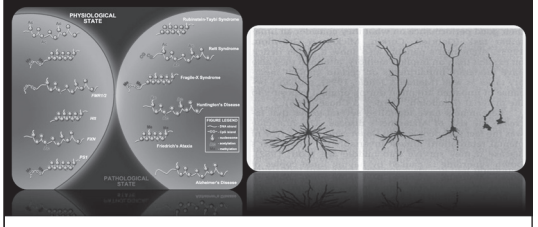

Neuronal Theory
Santiago Ramón y Cajal, sec. XIX.
MakeAGIF.com


Imaging techniques to study neuromorphology and physiology
Looping film of Neurons Firing
Sakaguchi Lab @ Iowa State
20 μm

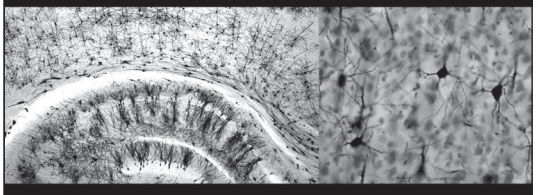
Imaging techniques to study neuromorphology and physiology

EM data from N. Kasthuri, R. Schalek, K. Hayworth, J.-C. Tapia, and J. Lichtman at Harvard University; reconstruction and rendering by D. Berger and S. Seung at MIT

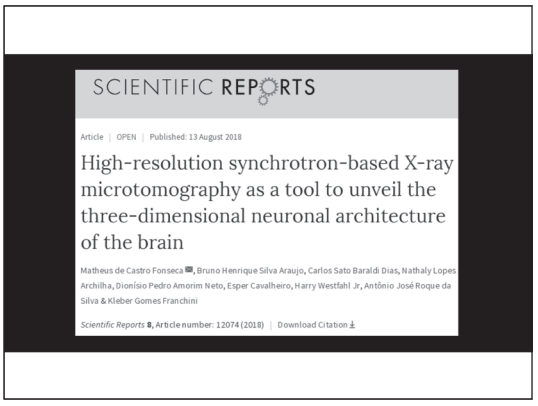
Morphofunctional alteration of the neuronal cell in neurodegenerative diseases

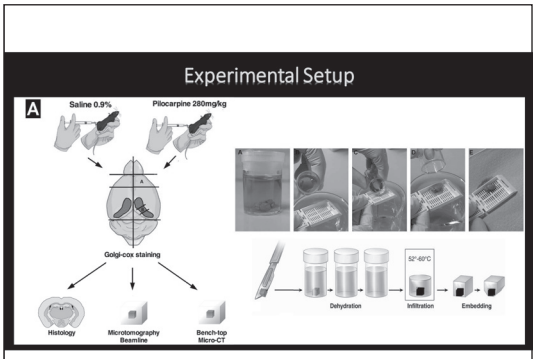


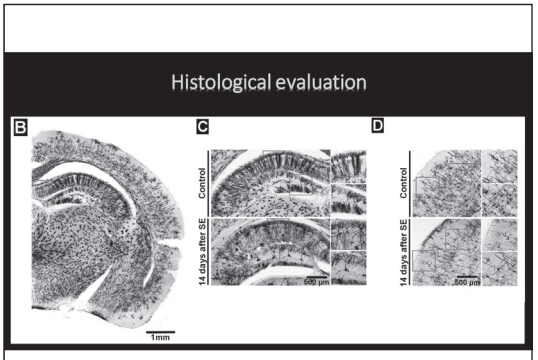
The Golgi-Cox technique

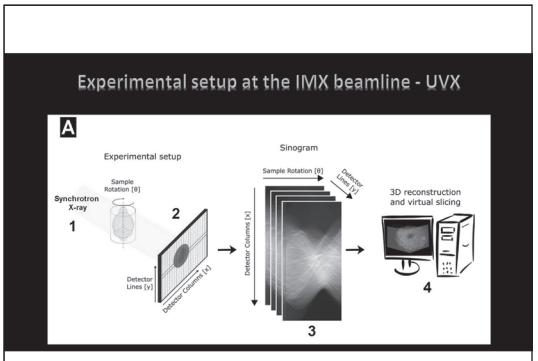


X-ray microtomography to study the nervous system in a macro and microscopic manner

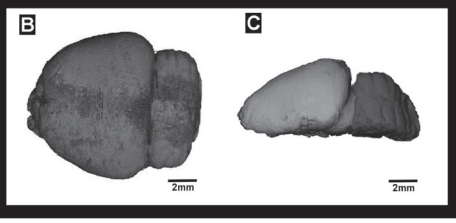

SCIENTIFIC REPORTS
Article | OPEN | Published: 13 August 2018
High-resolution synchrotron-based X-ray microtomography as a tool to unveil the three-dimensional neuronal architecture of the brain
Matheus de Castro Fonseca, Bruno Henrique Silva Araujo, Carlos Sato Baraldi Dias, Nathaly Lopes Archilha, Dionísio Pedro Amorim Neto, Esper Cavalheiro, Harry Westfahl Jr, Antônio José Roque da Silva & Kleber Gomes Franchini
Scientific Reports 8, Article number: 12074 (2018) | Download Citation


Experimental Setup
A
Saline 0.9%
Pilocarpine 280mg/kg
Golgi-cox staining
Histology
Microtomography Beamline
Bench-top Micro-CT
52°-60°C
Dehydration
Infiltration
Embedding

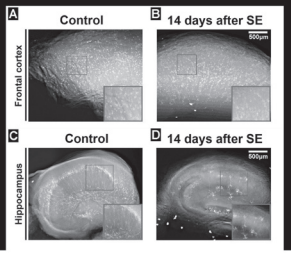

Histological evaluation
B
C
D
Control
14 days after SE
500 µm
1mm


Experimental setup at the IMX beamline - UVX
A
Experimental setup
Sinogram
Synchrotron X-ray
Sample Rotation [θ]
Detector Lines [y]
Detector Columns [x]
3D reconstruction and virtual slicing
1
2
3
4

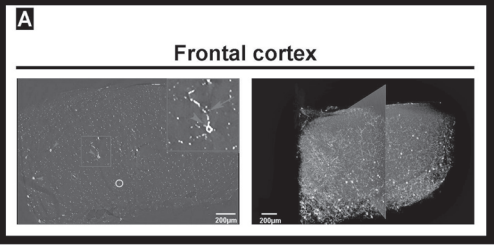
Whole brain tomography



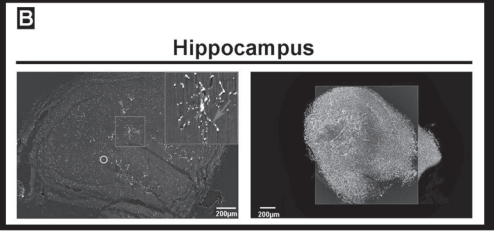
Cortex and Hippocampus projections– WT and SE

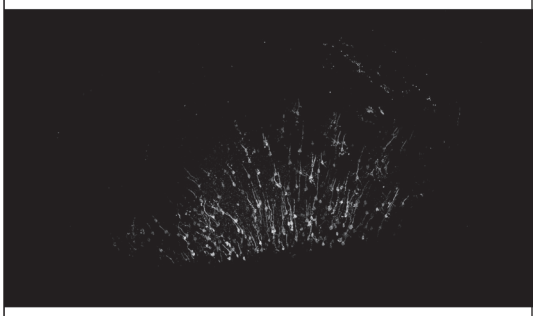


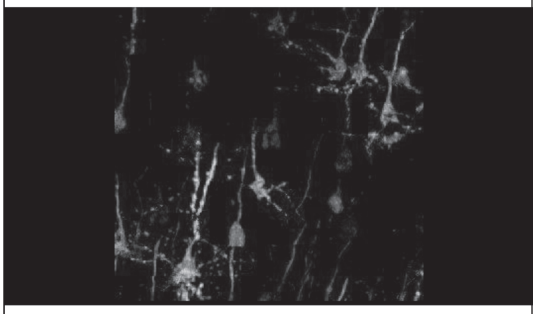
Automatic volume rendering: Cortex

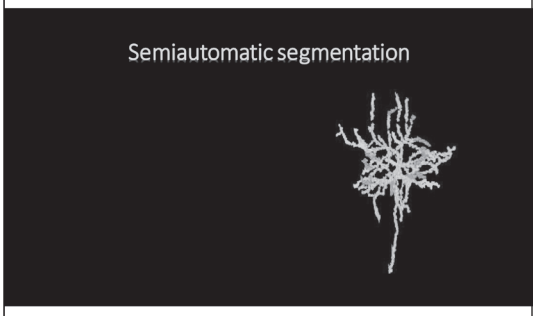


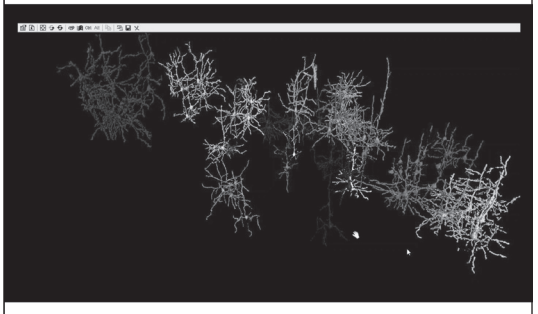
Automatic volume rendering: Hippocampus

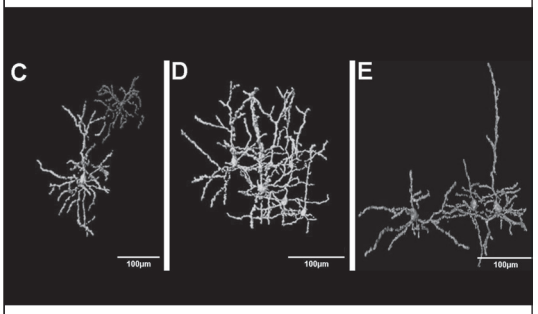


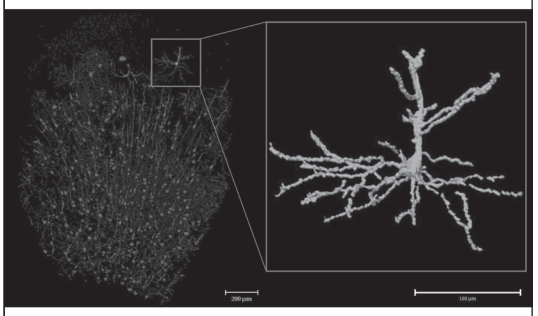


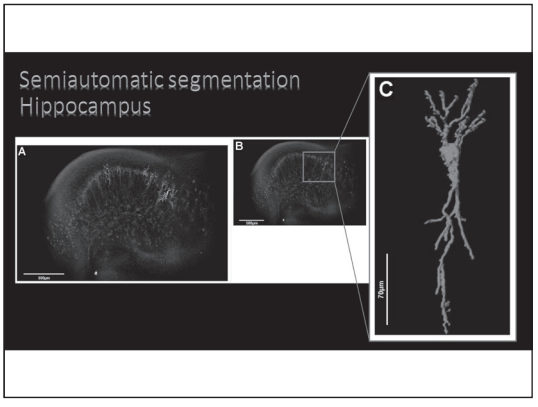


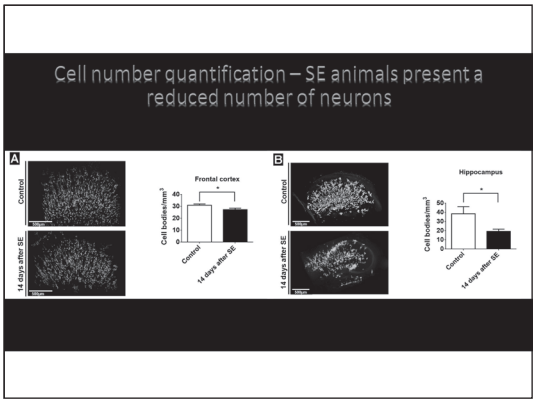

Semiautomatic segmentation



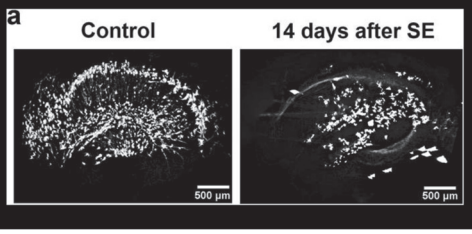

C
D
E
100µm
100µm
100µm


200 µm
100 µm

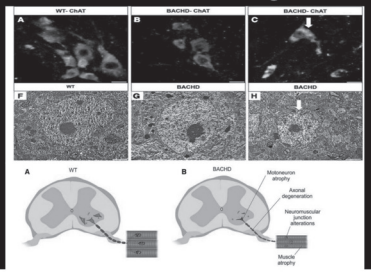

Semiautomatic segmentation
Hippocampus
A
B
C
70µm

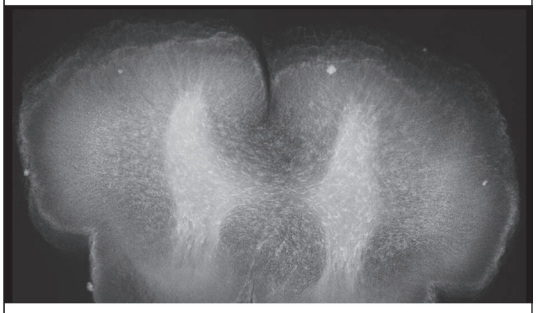

Cell number quantification – SE animals present a reduced number of neurons
A
Control
14 days after SE
Frontal cortex
Cell bodies/mm³
40
30
20
10
0
*
Control
14 days after SE
B
Control
14 days after SE
Hippocampus
Cell bodies/mm³
50
40
30
20
10
0
*
Control
14 days after SE

Microtomography at a bench-top µCT



BACHD mouse model of Huntington's disease





Take-home message

Synchrotron based X-ray microtomography is a powerful tool to study the nervous system at a macro and microscpic level
In health and disease.


Thanks
matheus.fonseca@lnbio.cnpem.br

Guilca Contreras (Venezuela)

NEUROSTIMULATION IN THE TREATMENT OF DRUG-RESISTANT EPILEPSY

GROUPS 2, 4, 6, 8

Maria Luiza Manreza (Brazil)

THE WIDE SPECTRUM OF METABOLIC ENCEPHALOPATIES

O amplo espectro das
Epilepsias "Metabólicas" da Infância

Dra Maria Luiza Manreza –
Neurologista Pediátrica

LBE

BiOMARIN

Conflito de Interesses

Pesquisadora Aché, UCB

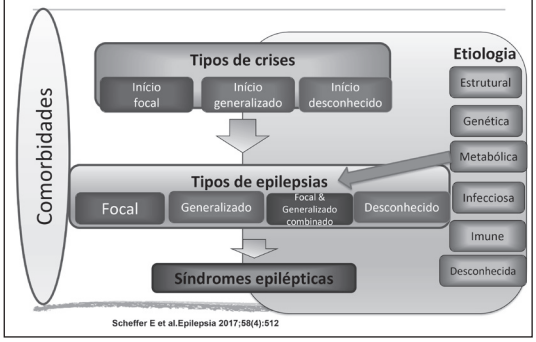
Palestrante: BioMarin e UCB

Consultora: BioMarin e UCB

Agenda

- ✓ **Encefalopatias epilépticas de etiologia metabólica**
  1. Definição
  2. Quando pensar em doença metabólica
  3. Principais encefalopatias epilépticas metabólicas
- ✓ **Lipofuscinose Ceroide Neuronal (LCN)**
  1. Definição e Histórico
  2. Incidência e Prevalência
- ✓ **Lipofuscinose Ceroide Neuronal Tipo 2 (CLN2)**
  3. Fisiopatologia
  4. Diagnóstico Clínico
  5. Exames complementares
  6. Tratamento da CLN2.
- ✓ **Considerações Finais**

CLASSIFICAÇÃO DAS EPILEPSIAS



Scheffer E et al.Epilepsia 2017;58(4):512

EPILEPSIA METABÓLICA

- ✓ Erros Inatos do Metabolismo (EIM) são uma causa rara de epilepsia, mas crises epilépticas e epilepsia são frequentemente encontradas em pacientes com EIM.

- ✓ EPILEPSIA METABÓLICA
- idade precoce de apresentação
- atraso / regressão do desenvolvimento
- resistência à terapia com FAE convencionais
- positividade em testes genéticos -> 7%
- condições tratáveis > 4%

Mercimek-Mahmutoglu S et al. Epilepsia. 2015; 56 : 707–716

ERROS INATOS DO METABOLISMO E EPILEPSIA FISIOPATOLOGIA

- ✓ **Interferem em funções do metabolismo cerebral**
- ✓ **Levam ao acúmulo de compostos que causam neurotoxicidade**
- ✓ **Determinam distúrbios primários ou secundários nas vias dos neurotransmissores**.
- ✓ **Associação à malformação do desenvolvimento cortical**
- ✓ **Outros mecanismos** como alteração na permeabilidade da membrana neuronal, deficiência de substrato, etc

Campistol J et Plecko B. Epileptic Disorder 2015; 17 : 229-242.

CLASSIFICAÇÃO NEONATAL E PRIMEIRA INFÂNCIA

- ✓ **INDÍCIOS DA PRESENÇA DE EIM NO PERÍODO NEONATAL**

- Consanguinidade parental
- História familiar de morte neonatal e/ou doenças neurológicas
- Gravidez - síndrome HELLP* , movimentos fetais excessivos (convulsões intra-uterinas)
- Deterioração após um período de aparente normalidade
- Encefalopatia Rapidamente Progressiva
- Acidose metabólica severa
- Soluços
- Odores incomuns de urina

*hemólise, elevação das enzimas hepáticas e plaquetas

Sharma S et Prasad AN.. Int J Mol Sci. 2017 Jul 2;18(7)

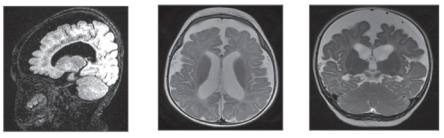
CLASSIFICAÇÃO NEONATAL E PRIMEIRA INFÂNCIA

- **Epilepsia Dependente de Piridoxina**
- Deficiência de piridox (am) ine 5 '-fosfato oxidase (PNPO)
- Convulsões responsivas ao ácido folínico
- Deficiência de biotinidase e holocarboxilase sintetase
- **Defeito do Transportador de Glicose (Síndrome de Deficiência de GLUT1)**
- Transtornos da Biossíntese Serina
- Cofactor de Molibdénio e Deficiência Isolada de Sulfito Oxidase
- Doença de Menkes
- Hiperglicinemia não-cetótica
- **Encefalopatia Epiléptica Responsiva à Uridina (CAD**)
- Defeitos do Ciclo da Ureia, Acidemias Orgânicas e Aminoacidopatias
- Transtornos Congênitos da Glicosilação
- Transtornos Peroxissômicos
- Transtornos Congênitos da Autofagia
- **Lipofuscinose Ceroide Neuronal**

Sharma S et Prasad AN. Int J Mol Sci. 2017 Jul 2;18(7).

CASO CLÍNICO 1

- ✓ Criança do sexo feminino, 11 meses de idade
- ✓ Sem relato de consanguinidade, mas **história familiar de irmã com quadro similar**, que foi a óbito aos 2 anos de idade por provável SUDEP
- ✓ Referia crises epilépticas desde o 1º dia de vida, "tremores generalizados" inicialmente controladas com fenobarbital, mas que evoluíram com diversas semiologias inclusive espasmos epilépticos, descompensações graves, EME, fármaco-resistência e regressão do DNPM
- **EEG**: multifocal, status, surto supressão



CASO CLÍNICO 1

✓ EXOMA

Exame: Sequenciamento do exoma

Resultado

Diagnóstico: Epilepsia Piridoxina-dependente (OMIM # 266100)

Gene	Posição	Variação	Consequência	Cópias	
ALDH7A1	chr5:125.894.936	C > T	p.Arg335Gln ENST00000409134	Homozigose (2 cópias)	5

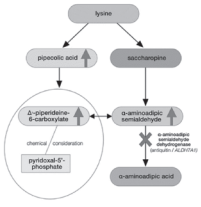
5 Definitivamente patogênico

Método

Captura de exons com Agilent SureSelect Clinical Research Exome V2 seguida por sequenciamento de nova geração com Illumina HiSeq. Alinhamento e identificação de variantes utilizando protocolos de bioinformática, tendo como referência a versão GRCh37 do genoma humano. Análise médica orientada pelas informações que motivaram a realização deste exame.

Epilepsia Dependente de Piridoxina

METABOLISMO DA LISINA



Mutação do gene ***ALDH7A1***

✓ Deficiência de semi-desidrogenase α-aminoadípica (**antiquitina**), resulta no acúmulo de :

1. semialdeído α-aminoadípico (AASA)
2. Δ-1-piperidina-6-carboxilato (P6C)
3. ácido pipecólico

Van Karnebeek CD et Jaggumantri S. Curr Treat Options Neurol 2015;17:335.

Epilepsia Dependente de Piridoxina

- ✓ Doença autossômica recessiva rara: prevalência de 1: 20.000 a 1: 600.000
- ✓ Caracterizada por convulsões recorrentes nos períodos pré-natal, neonatal e/ou pós-natal resistentes aos fármacos antiepilépticos (FAE) convencionais, mas responsiva às dosagens farmacológicas da piridoxina
- ✓ Formas típicas: as crises iniciam-se horas ou dias após ou mesmo intra-útero
- ✓ Formas atípicas:
  - início tardio
  - crianças cujas convulsões respondem inicialmente aos FAEs, mas recorrem semanas a meses mais tarde de forma refratárias
  - pacientes cujas convulsões não são controladas por grandes doses iniciais de piridoxina, mas que respondem mais tarde a um segundo tratamento

Van Karnebeek CD et Jaggumantri S. Curr Treat Options Neurol 2015;17:335.

Epilepsia Dependente de Piridoxina Características

- ✓ **Sintomas gerais** iniciais: vômitos, distensão abdominal, insônia, irritabilidade, contratura facial paroxística e movimentos oculares anormais.
- ✓ E**pilepsia** é bastante variável e dependente da resposta a piridoxina ocorrem crises: focais motoras recorrentes, tônicas generalizadas, mioclonicas, espasmos infantis, convulsões e estado epiléptico recorrente
- ✓ **EEG** é inespecífica e pode permanecer anormal mesmo com a terapia com piridoxina
- ✓ **Retardo do desenvolvimento neuropsicomotor** varia de leve a grave. A incapacidade intelectual comumente afeta a linguagem expressiva, juntamente com um QI de desempenho / motor abaixo do normal
- ✓ **Ressonância magnética,** padrões variáveis , desde lesões da substância branca, atrofia cerebral geral, hipoplasia ou displasia do corpo caloso até um cérebro estruturalmente normal

Van Karnebeek CD et Jaggumantri S. Curr Treat Options Neurol 2015;17:335.

Epilepsia Dependente de Piridoxina Tratamento

- ✓ DOSE INICIAL: 100 mg EV
  - Sem resposta: doses adicionais 100 mg
  - Até total de 500 mg
  - Na dúvida sobre uma resposta parcial: manter 15-30 mg/kg/dia por 7 dias
- ✓ MANUTENÇÃO
  - Suplementação diária de piridoxina por toda a vida: 5 a 15 mg/kg/dia
  - Em geral, 50-100 mg/dia
  - Máx: 200 mg em crianças e 500 mg em adultos
  - Uso excessivo de piridoxina neuropatia sensorial reversível, apneia e hipotonia
- ✓ **Tratamento pré-natal** – dose de 100 mg de piridoxina /dia parece ser segura
- ✓ Ácido folínico falta de resposta á piridoxina (3-5mg/kg/d a 10-30mg/d)
- ✓ Dieta com restrição de lisina- difícil, poucos estudos

Van Karnebeek CD et Jaggumantri S. Curr Treat Options Neurol 2015;17:335.

Epilepsia Dependente de Piridoxina Fenótipo - Evolução

- ✓ Os fenótipos clínicos de epilepsia dependente de piridoxina podem ser classificados em três grupos:
  - Grupo 1 consiste em pacientes com controle completo das crises, com piridoxina e desenvolvimento normal
  - **O grupo 2 consiste em pacientes com controle completo das crises com piridoxina, mas com atraso no desenvolvimento**
  - Grupo 3 consiste em pacientes com convulsões persistentes e atraso no desenvolvimento, apesar da piridoxina.
- ✓ Apesar de extensos estudos, as correlações entre o genótipo e essas categorias fenotípicas ainda não foram estabelecidas

Van Karnebeek CD et Jaggumantri S. Curr Treat Options Neurol 2015;17:335.

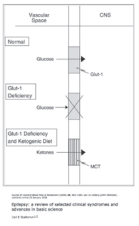
CASO CLÍNICO 2

- Criança do sexo masculino com 8 meses de idade
- Filha de pais não consanguíneos, sem antecedentes pré e perinatais importantes.
- Aos 4 meses a mãe observou que não interagia com o meio. Nesta mesma ocasião percebeu movimentos rotatórios dos olhos e crises epilépticas caracterizadas desvio dos olhos e da cabeça para a esquerda, nistagmo e postura tônica dos MMSS, com duração de 5-10s, cerca de 20x ao dia. Utilizou fenobarbital clonazepam sem controle das crises .

- Exames
- **Glicemia – 98 mg/dL**
- **LCR – glicose 38 mg/dl**
- **Relação glicose LCR/glicose sangue: 0,38 (N=1,1-0,4 ou 0,6)**
- Exame genético confirmou o diagnóstico

Síndrome de Deficiência de GLUT1
Defeito do Transportador de Glicose
(Síndrome de De Vivo)

- ✓ O cérebro é dependente de glicose existindo nove transportadores para vencer a barreira hemato-encefálica incluindo o GLUT1
- ✓ O gene *SLC2A1* (1p35-31.3) codifica o GLUT 1
- ✓ A deficiência de Glut-1 é geralmente autossômica dominante mas têm sido descritos casos de herança autossômica recessiva

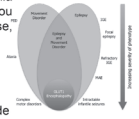


Journal of Neurophysiology 8:1-9, Copyright © 2010 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.
Epilepsy: a review of selected clinical syndromes and advances in basic science
Carl E. Stafstrom CT

Kass HR, et al. Seizure 2016 Feb;35:83

Síndrome de Deficiência de GLUT1
Defeito do Transportador de Glicose

- ✓ **Síndrome de deficiência de GLUT1**
- Crises refratárias de início precoce, RDNPM, microcefalia adquirida, anormalidades do tônus muscular (hipotonia ou espasticidade) e distúrbios do movimento, como coreoatetose, ataxia e distonia
- ✓ **O espectro clínico** da deficiência de GLUT1 é mais amplo:
- RDNPM, epilepsia e formas familiares e esporádicas de discinesia induzida por exercício paroxístico
- Diferentes graus de comprometimento cognitivo associados a disartria, disfluência e déficits de linguagem expressiva são características adicionais



Neurology 2010; 75: 632

Kass HR, et al.. Seizure. 2016 Feb;35:83

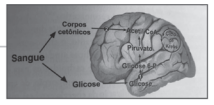
Paroxysmal eye-head movements in Glut1 deficiency syndrome.

Estudo retrospectivo do primeiro evento neurológico (Columbia University n = 133)
- ✓ Crise epiléptica – 61%
- ✓ Movimentos oculares anormais – 38%
- "rolar os olhos", "tremor dos olhos", "opsoclonus"
- 85% apresentaram os primeiros movimentos oculares em torno de 6 meses

- Os movimentos oculares são sacádicos e geralmente conjugados
- cada três desvios de olhar, é seguido por um movimento da cabeça na mesma direção.
- Existem períodos de fixação entre as sacadas

Neurology. 2017 Apr 25;88(17): 1666-1673

Síndrome de Deficiência de GLUT1



TRATAMENTO

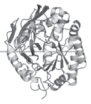
- ✓ Dieta cetogênica
- Rica em gorduras, adequada em proteínas e pobre em carboidratos
- Determina cetose, mimetizando o jejum
- Dieta cetogênica clássica: 4gr de gordura, 1gr de carboidrato e proteína

- ✓ FISIOPATOLOGIA: fonte alternativa de energia para o cérebro
- Boa adesão
- Controle eficiente de crises
- Melhor do alerta e da atividade
- Em alguns casos pode ser necessário manter os FAE

- ✓ Criança citada iniciou tratamento com dieta cetogênica
- **Evoluiu com controle das crises epilépticas e melhora do desenvolvimento**.
- Iniciado redução PB.

Kass HR, et al. Seizure. 2016 Feb;35:83
Sampaio LPB. Tratamento medicamentoso das epilepsias /ed Yacubian/Contreras-Caicedo/ Rios-Pohl , 2014 pg207

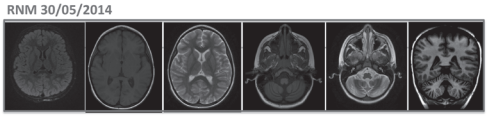
CASO CLÍNICO 3



Caso Clínico

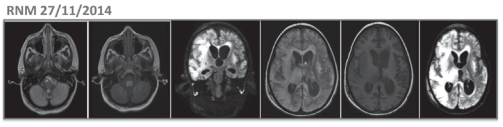
- ✓ Criança do sexo feminino, 8 anos, sem antecedentes perinatais DNPM: adequado, exceto por discreto atraso da linguagem
- ✓ Aos 13 meses de idade com história de **anemia** iniciou crises epilépticas focais motoras disperceptivas frequentes e refratárias a vários esquemas terapêuticos. Seguiu-se involução do DNPM, aparecimento de crises generalizadas sendo internada em mais de uma ocasião em estado de mal epiléptico. Persistiu piorando necessitando de traqueostomia.
- ✓ LCR - **aumento de lactato**
- ✓ EEG - desorganização difusa da atividade elétrica cerebral, por maior teor de ondas lentas teta; paroxismos de espículas e ondas agudas, de projeção centroparietal bilateral, com difusão para as regiões anteriores
- ✓ HD- MELAS? Lipofuscinose ceroide neuronal?

RNM 30/05/2014



- Proeminências dos espaços liquóricos ao redor do cerebelo, sugerindo redução volumétrica
- Espectroscopia com redução da relação NAA/Cr na substancia branca e cinzenta parieto-occipital podendo representar perda e/ou disfunção neuroaxonal

RNM 27/11/2014



- Redução volumétrica encefálica, múltiplas lesões cortico-subcorticais nos hemisférios cerebrais e cerebelares, na substancia branca periventricular, região nucleocapsular e tálamos.
- Espectroscopia com aumento do lactato
- Lesões compatíveis com insultos isquêmicos em diferentes territórios e fases evolutivas, podendo-se considerar as possibilidade de hipóxia secundaria ao referido ou mitocondriopatia.

Encefalopatia Epiléptica Responsiva à Uridina (CAD)

- ✓ Distúrbio por mutações do *CAD**
- *CAD* codifica uma enzima multifuncional (CPSase / ATCase / DHOase) envolvida na biossíntese de novo de pirimidina.
- As pirimidinas também podem ser recicladas a partir da uridina.

- ✓ Características clinicas
  - Atraso / regressão do desenvolvimento neuropsicomotor
  - Encefalopatia epiléptica grave
  - Aos 4 anos de idade – restritos ao leito, rebaixamento do nível de consciência
  - **Anemia**

- ✓ Literatura refere quatro crianças com RDNPM, encefalopatia epiléptica e anemia
- Duas faleceram pós um curso de 4-5 anos de doença neurodegenerativa
- Duas receberam suplementação com uridina oral: cessação imediata das crises melhora dramática cognitiva e motora e resolução da anemia.

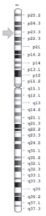
*CAD protein (carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase)

Koch J et al. Brain. 2017 Feb;140(2):279

Encefalopatia Epiléptica Responsiva à Uridina (CAD)

- ✓ EXOMA- Duas variantes em heterozigose no gene CAD
  - Chr 2:27.445.462 C>T -> Substituição da arginina na posição 191 por um códon de parada. Definidamente patogênica
  - Chr 2:27.462.599 G>A -> Substituição da arginina na posição 1810 por glutamina. Provavelmente patogênica

- ✓ Tratamento via oral com uridina controle da anemia e melhora da epilepsia



CASO CLÍNICO 4

- ✓ AF– Pais consanguíneos. Irmã com quadro semelhante diagnosticada quando o paciente já tinha a doença
- ✓ DNPM – normal até 4 anos de idade exceto discreto atraso de linguagem
- ✓ Aos 4 anos iniciou com ataxia e mioclonias resitentes aos tratamentos evoluindo com piora progressiva dos sintomas e involução do DNPM deixando progressivamente de sentar, andar e falar. Ficou restrito ao leito persisitindo as mioclonias. Evoluiu para óbito aos 8,5 anos de idade

4 anos

4 anos 6 meses

6 anos

Youtube - CLN2 Batten Disease progression - Noah's Hope

Frederick Batten
1903

Lipofuscinose Ceroide Neuronal
DOENÇA DE BATTEN

Frequência

- ✓ Uma das doenças neurodegenerativas herdadas mais comuns da infância
- ✓ Prevalência e incidência dependem de ascendência e região geográfica:
  - Prevalência (/população) → 1:1.000.000 em algumas regiões a 1:100.000 nos países escandinavos;
  - Incidência (/nascidos vivos) → 1:67.000 (Itália e Alemanha) a 1:14.000 (Islândia).
- ✓ 13 genes descritos e mais de 500 mutações listadas
- ✓ 14 formas da doenças
- ✓ **As mais prevalentes são: CLN3 - fenótipo juvenil clássico, seguido por CLN2 - fenótipo infantil tardio**.

Schulz A, et al. Biochim Biophys Acta. 2013;1832:1801-1806; Haltia M et Goebel HH. Biochim Biophys Acta. 2013; 1832: 1795; Williams RE. : NCL incidence and prevalence data. In: The Neuronal Ceroid Lipofuscinoses (Batten Disease). 2nd edition; Mole SE, Williams RE, Goebel HH (Eds). Oxford: Oxford University Press; 2011: pp. 361-363; Simonati et al., Current Molecular Medicine 2014, 14, 1043-1051

Classificação e características das LCNs

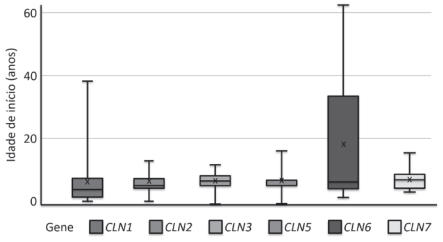
Doença*	Manifestação e fenótipo clínico	Gene	Proteína
CLN1	Clássica infantil, infantil tardio, juvenil, adulto	*CLN1 (PPT1)*	Proteína palmitoil tioesterase
CLN2	Fenótipo clássico infantil tardio; fenótipos atípicos: infantil, juvenil, prolongado; *SCAR7* ↑	***CLN2 (TPP1)***	Tripeptidil peptidase-1
CLN3	Clássico juvenil	*CLN3*	Proteína transmembranar
CLN4	Adulto (autossômico dominante)	*CLN4 (DNAJC5)*	Proteína da cadeia de cisteína solível
CLN5	Variante infantil tardio, juvenil, adulto	*CLN5*	Proteína solível lisossomal
CLN6	Variante infantil tardio, adulto (Kufs tipo A)	*CLN6*	Proteína transmembranar
CLN7	Variante infantil tardio	*CLN7 (MFSD8)*	Proteína transmembranar
CLN8	Variante juvenil tardio	*CLN8*[a]	Proteína transmembranar
CLN9	Variante juvenil	—	—
CLN10	Clássico congênito, infantil-tardio, juvenil, adulto	*CLN10 (CTSD)*	Catepsina D
CLN11	Adulto	*CLN11 (GRN)*	Progranulina
CLN12	Juvenil	*CLN12 (ATP13A2)*[a]	ATPase
CLN13	Adulto (Kufs tipo B)	*CLN13 (CTSF)*	Catepsina F
CLN14	Infantil	*CLN14 (KCTD7)*[a]	Proteína do canal de potássio

* Doenças LCN conhecidas. Os genes de LCN adicionais ainda devem ser identificados.

Schulz A, et al. *Biochimica et Biophysica Acta*. 2013;1832:1801-1806. OMIM database. TPP1; http://www.omim.org/entry/607998. Accessed July 14, 2016. OMIM database. Kufor-Rakeb Syndrome; http://www.omim.org/entry/606693. Accessed July 14, 2016. OMIM database. EPM3; http://www.omim.org/entry/611726. Accessed July 14, 2016.

Classificação e características das LCNs

Idade de início em pacientes com LCNs por genótipo *(não foram descritos os genótipos com <5 indivíduos)*



Adaptado de: G. Aungaroon et al. / Pediatric Neurology 60 (2016) 42e48

Lipofuscinose Ceroide Neuronal TIPO 2 CLN2

Doença	Epônimo	Nº OMIM	Fenótipo	Nome abreviado
CLN2	Jansky-Bielschowsky	204500	Infantil tardia clássica Juvenil	LINCL

Cromossoma	Gene	Produto do Gene	Proteína Acumulada
11p15	*TPP1/CLN2*	TPP1	SCMAS

Lipofuscinose Ceroide Neuronal Tipo 2 (CLN2)

- ✓ Um subtipo da doença de Batten
- ✓ Herança autossômica recessiva (gene *CLN2*)
- ✓ É uma das doenças de depósito lisossômico
- ✓ Incidência estimada: 0,22 a 9/100.000 nascidos vivos dependendo da região geográfica
- ✓ A neurodegeneração é o resultado da deficiência ou ausência da atividade da enzima TPP1
- ✓ A ausência ou atividade reduzida da enzima TPP1 é associada ao acúmulo de lipopigmento autofluorescente lisossômico (lipofuscina ceroide)
- • Com o tempo, ocorre disfunção e morte celular, atrofia neuronal e da retina.

Chang M, et al. CLN2. In: Mole S, Williams R, and Goebel H, eds. The neuronal ceroid lipofuscinoses (Batten Disease). 2nd ed. Oxford, United Kingdom: Oxford University Press; 2011:80-109. Haltia M. *Biochim Biophys Acta*. 2006;1762:850-856. Anderson GW, et al. *Biochim Biophys Acta*. 2013;1832:1807-1826.

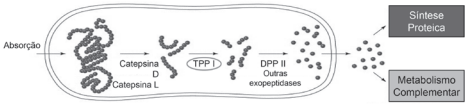
FISIOPATOLOGIA
ATIVIDADE ENZIMÁTICA

- ✓ CLN-> 4 enzimas lisossomais foram identificadas como deficientes em leucócitos, fibroblastos e vilo corial:
- Palmitoil-proteína tioesterase 1 (PPT 1) codificada por *PPT1 (CLN1)*
- **Tripeptidil-peptidase 1 (TPP-1) codificado por *TPP1* (*CLN2*)**
- Catepsina D (CTSD) codificado por *CTSD (CLN10)*
- Catepsina F (CTSF) codificado por *CTSF (CLN13)*

Neverman NJ et al. Biochim Biophys Acta 2015; 6:S925

Envolvimento da Tripeptidil-peptidases na degradação proteica

Degradação de proteína lisossomal



atividade deficiente da enzima TPP1 | acúmulo de lipopigmento autofluorescente lisossômico. | disfunção, morte e atrofia celular.

Adaptado de Tomkinson B, Tripeptidyl Peptidades: Enzymes That Counts, TIBS 24 – SEPTEMBER 1999, p355-356, PII: S0968-0004(99)01435-8

Lipofuscina

- ✓ Lipopigmento autofluorescente de origem glicoproteica
- ✓ Pigmento fino, castanho-dourado, que resulta da digestão incompleta dos glóbulos sanguíneos danificados (resíduos celulares)
- ✓ Este pigmento serve para detectar o tempo de vida celular. Ele está presente em células que não se multiplicam muito e têm vida longa, como as musculares do miocárdio e os neurônios
- ✓ Normalmente, quanto mais lipofuscina presente, mais velha é a célula
- ✓ Ceroide – que tem aspecto de cera

Brunk UT1, Terman A, Free Radic Biol Med. 2002 Sep 1;33(5):611-9.

Lipofuscinose Ceroide Neuronal Tipo 2 (CLN2)
Diagnóstico Clínico

A doença CLN2 é uma patologia de progressão rápida que apresenta um conjunto de sintomas

- ✓ A**traso de linguagem** recentemente identificado como um sinal inicial da doença CLN2, na maioria dos pacientes.
- ✓ **Crises epilépticas** de início entre as idades de 2 e 4 anos no fenótipo clássico
- ✓ **Perda completa das habilidades cognitivas, função motora e visão**:
  - A perda da visão ocorre com o avanço da doença.

Exemplo de progressão da doença CLN2

18 meses | 3 anos de idade | 5 anos de idade | 7 anos de idade | 10 anos de idade

Nickel M, et al. Poster session presented at: The 12th Annual WORLD Symposium; February-March 2016; San Diego, CA; Mole SE, et al. *Neurogenetics*. 2005;6:107-126.; Schulz A, et al. *Biochim Biophys Acta*. 2013;1832:1801-1806; Mole SE, et al. 2001 Oct 10 [Updated 2013 Aug 1]. In: Pagon RA, et al., eds. GeneReviews; Steinfeld R, et al. *Am J Med Genet*. 2002;112:347-354.

CLN2- DIAGNÓSTICO
Atraso Precoce de Linguagem

- ✓ 83% das crianças com a doença CLN2 apresentam atraso precoce de linguagem:
  - Menos de 25% das crianças entre 2 a 4 anos de idade que procuram cuidados médicos com crises epilépticas não provocadas de início recente também apresentam atraso da linguagem;
  - Em alguns casos, também pode ocorrer ataxia ou outras alterações do desenvolvimento, ou esses podem ser os primeiros sinais da doença CLN2.

Critérios para identificar atraso precoce de linguagem:

- **Primeiras palavras isoladas aos 18 meses (ou mais tarde/nunca)**
- **Primeiras frases de duas palavras aos 24 meses (ou mais tarde/nunca)**
- **Primeiras frases completas aos 36 meses (ou mais tarde/nunca)**

Nickel M, et al. Poster session presented at: The 12th Annual WORLD Symposium; February-March 2016; San Diego, CA.; Andell E, et al. *Epilepsy Res*. 2015;113:140-150; Worgall S, et al. *Hum Gene Ther*. 2008;19:463-474.; Chang M, et al. CLN2. In: Mole S, Williams R, and Goebel H, eds. The neuronal ceroid lipofuscinoses (Batten Disease). 2nd ed. Oxford, United Kingdom: Oxford University Press; 2011:88-109.

CLN2 - DIAGNÓSTICO
Crises Epilépticas Não Provocadas De Início Precoce

- ✓ Na maioria das crianças com a doença CLN2, crises epilépticas não provocadas surgem entre os 2 e 4 anos de idade;
- ✓ Embora as crises epilépticas sejam frequentemente não provocadas, podem ocorrer crises febris;
- ✓ Mioclonias - tanto epilépticas quanto não epilépticas – são predominantes;
- ✓ Crises tônico-clônicas generalizadas, de ausência, clônicas, tônicas e atônicas também podem ocorrer;
- ✓ As crises epilépticas persistem durante o curso da doença e são refratárias ao tratamento.

Schulz A, et al. *Biochim Biophys Acta*. 2013;1832:1801-1806; Schulz A, et al. Poster session presented at: The Society for the Study of Inborn Errors of Metabolism (SSIEM) Annual Symposium; September 2015; Lyon, France.

CLN2- DIAGNÓSTICO
Características Da Involução Psicomotora

- ✓ A involução pode ser descrita em fases:
  - 1ª fase → perda das sentenças, geralmente aos 3 anos de idade;
  - 2ª fase → perda da capacidade de andar e da comunicação verbal;
  - 3ª fase → perda da capacidade de sentar, do uso das mãos, do controle esfincteriano. Ao redor de 5 anos de idade tornam-se cadeirantes e, em meses, disfágicos.

Pérez-Poyato MS et al. J Child Neurol 2013;28(4):470

CLN2- DIAGNÓSTICO
Outros Sintomas Neurológicos

- ✓ Ataxia;
- ✓ Espasticidade;
- ✓ Movimentos involuntários:
  - Mioclonias (epilépticas e não epilépticas) são uma das características da CLN2;
  - Distonia e espasticidade também são achados comuns; coreia, atetose e tremores também podem ser vistos. *Status distonicus* e *status* mioclônico podem ser complicações com risco de vida;
  - Parkinsonismo, ataxia e coreia proeminentes têm sido relatados em fenótipos atípicos.

Chang M, et al. CLN2. In: Mole S, Williams R, and Goebel H, eds. The neuronal ceroid lipofuscinoses (Batten Disease). 2nd ed. Oxford, United Kingdom: Oxford University Press; 2011:80-109. Perez-Poyato MS, et al. *J Child Neurol.* 2012;28:470-478.; Steinfeld R, et al. *Am J Med Genet.* 2002;112:347-354; Schulz A, et al. *Biochim Biophys Acta.* 2013;1832:1801-1806. Worgall S, et al. *Hum Gene Ther.* 2008;19:463-474.

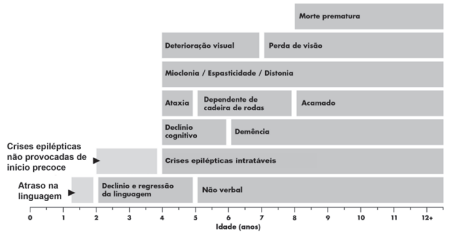
CLN2- DIAGNÓSTICO
Diagnóstico visual

- ✓ Perda de visão na CLN2 ocorre secundária a degeneração progressiva da retina de fisiopatologia incerta;
- ✓ As manifestações oftalmológicas da CLN2 estão intimamente relacionadas com o grau de função neurológica e a idade do paciente, sendo úteis na avaliação de novas estratégias terapêuticas;
- ✓ **Avaliação:**
  - Potencial visual evocado: aumento da latência. Intensificado cedo na doença, mas reduzido no estágio final da doença;
  - Tomografia de coerência óptica: Avalia a progressão através de degeneração da retina (ex.: acúmulo de material hiperrefletivo, afinamento da retina com perda de fotorreceptores, aspecto de olho de boi);
  - Eletrorretinograma: Pode ser reduzido antes da deterioração visual ser clinicamente detectada.

Chang M, et al. CLN2. In: Mole S, Williams R, and Goebel H, eds. The neuronal ceroid lipofuscinoses (Batten Disease). 2nd ed. Oxford, United Kingdom: Oxford University Press; 2011:80-109; Mole SE, et al. Neurogenetics. 2005;6:107-126;
Orlin A, et al. PLoS One. 2013;8:e73128; Mole SE, et al. 2001 Oct 10 [Updated 2013 Aug 1]. In: Pagon RA, et al., eds. GeneReviews.

Crianças apresentam perda significativa das funções à medida que os sintomas evoluem com a idade

Linha do tempo da manifestação de sintomas da doença CLN2 clássica



Chang M, et al. CLN2. In: Mole S, Williams R, and Goebel H, eds. The neuronal ceroid lipofuscinoses (Batten Disease). 2nd ed. Oxford, United Kingdom: Oxford University Press; 2011:80-109. Perez-Poyato MS, et al. *J Child Neurol.* 2012;28:470-478.; Steinfeld R, et al. *Am J Med Genet.* 2002;112:347-354; Schulz A, et al. *Biochim Biophys Acta.* 2013;1832:1801-1806.Worgall S, et al. *Hum Gene Ther.* 2008;19:463-474.

[redacted]

FORMAS ATÍPICAS DA DOENÇA CLN2

- ✓ Os fenótipos atípicos são mais raros, caracterizados por idades de início variadas e/ou maior expectativa de vida, como por exemplo:
  - Forma Juvenil: evolução mais benigna, predomínio de sintomas extrapiramidais como coreia, distonia, parkinsonismo;
  - Forma do Adulto: variante de ataxia espinocerebelar 7 (SCAR7), pode não apresentar epilepsia.

Fietz M et al. Mol Genet Metab. 2016 ;119:160;
Kohan R, et al. Biochim Biophys Acta. 2015;1852:2301

Lipofuscinose Ceroide Neuronal Tipo 2 (CLN2)

Exames Complementares

O eletroencefalograma (EEG)

- ✓ EEG é o primeiro exame que deve ser realizado;
- ✓ Atividade de fundo alentecida (pode ser normal na 1ª fase da doença), com alterações epileptiformes generalizadas que predominam nas áreas posteriores;
- ✓ Fotoestimulação intermitente (FEI) a 1-2Hz deve ser solicitada sempre;
- ✓ É importante testar todas as frequências para não perder as características informativas do EEG.

Fietz M et al. Mol Genet Metab 2016;119:160-167

4

Como solicitar o EEG

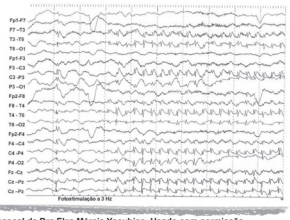
Solicito:

EEG em vigília, sonolência e sono com estimulação fótica enfatizando as frequências baixas de fotoestímulo - 0,5/s; 1/s; 2/s; 3/s

Arquivo pessoal da Dra Elza Márcia Yacubian. Usado com permissão.

Fotostimulação de baixa frequência

- ✓ Pontas gigantes occipitais em paciente com a doença CLN2
  - Ocorrência de potenciais evocados gigantes com maior amplitude nas regiões occipitais, em resposta ao estímulo fótico de 3 lampejos/seg., com frequência idêntica à estimulação.

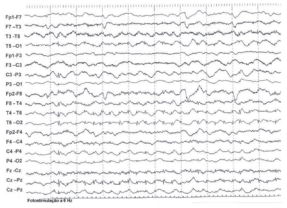


Arquivo pessoal da Dra Elza Márcia Yacubian. Usado com permissão.

4

Fotostimulação de baixa frequência

- ✓ Pontas gigantes occipitais em paciente com doença CLN2
  - Estes potenciais apresentam redução em amplitude e menor correlação com os lampejos conforme a frequência dos últimos é aumentada.



Arquivo pessoal da Dra Elza Márcia Yacubian. Usado com permissão.

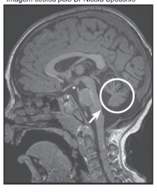
Photosensitivity is an early marker of neuronal ceroid lipofuscinosis type 2 disease (Specchio N, et al., Epilepsia 2017 Jun 20)

- ✓ N = 14 pacientes, 2005-2015. Idade de início aos 3,0 (2,0-3,8) anos;
- ✓ Sintomas:
  - Atraso ou regressão do DNPM, 100% aos 3 anos de idade;
  - Início da epilepsia (50%) aos 3,2 anos de idade (2,6-3,8) e a primeira crise foi:
    - Generalizada: mioclônica em 36%, TGC em 29%, atônica em 22%;
    - Focal com sinais motores em 14%;
    - Marcha independente, 100% aos 12 meses de idade.
- ✓ Exames:
  - EEGs com resposta fotoparoxística → 93%;
  - Atrofia cerebelar na RM → 100%;
  - Alteração da substância branca na RM → 79%.

Achados de ressonância magnética

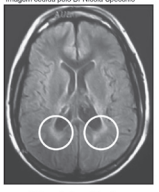
RM de paciente CLN2

Imagem cedida pelo Dr Nicola Specchio



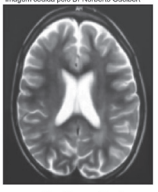
Atrofia Cerebelar

Imagem cedida pelo Dr Nicola Specchio



Hiperintensidades periventriculares na substância branca

Imagem cedida pelo Dr Norberto Guelbert



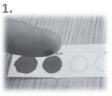
Atrofia cerebral

Chang M, et al. CLN2. In: Mole S, Williams R, and Goebel H, eds. The neuronal ceroid lipofuscinoses (Batten Disease). 2nd ed. Oxford, United Kingdom: Oxford University Press; 2011:80-109. 2. Mole SE, et al. Neurogenetics. 2005;6:107-126.

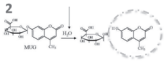
DOSAGEM DA ATIVIDADE DA ENZIMA TPP1

- ✓ CLN2: deficiência na enzima tripeptidil-peptidase 1 (TPP1);
- ✓ **Processo:**
  1. A atividade pode ser medida em leucócitos, fibroblastos, vilosidades coriônicas e fluido amniótico;
     - Adaptado para gota de sangue seca (*DBS*).
  2. A enzima TPP1 cliva o substrato artificial em pH ácido;
  3. Composto fluorogênico 7-amino-4-metilcumarin é liberado e medido no fluorímetro.

1.



2



3

Imagens de propriedade da BioMarin.

Miller et al. Poster presented at ASHG 2016; Fietz M, et al. Mol Genet Metab. 2016;119:160-167; Giugliani R, et al. Mol Genet Metab. 2016;117:S61, Abstract 143.

SEQUENCIAMENTO DO GENE *CLN2*/*TPP1*

- ✓ O gene *CLN2/TPP1* é localizado no cromossomo 11p15.4, contém 13 éxons e tem 6,7kbp de comprimento;

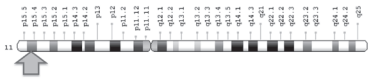


Imagem sem direitos autorais.

- ✓ Sequenciamento de todos os éxons e regiões flanqueadoras associadas para detecção de variantes patogênicas (*SNPs* e pequenos *indels*);
  - *Next generation sequencing (NGS)* → Técnica padrão;
  - *Sanger sequencing* → Confirmar variantes novas e regiões perdidas.
- ✓ Importante confirmar que variantes patogênicas sejam encontradas nos dois alelos (em homozigose ou heterozigose composta em *trans*).

Miller et al. Poster presented at ASHG 2016; Fietz M, et al. Mol Genet Metab. 2016;119:160-167; Giugliani R, et al. Mol Genet Metab. 2016;117:S61, Abstract 143.

Orientação genética e planejamento familiar

- ✓ Doença com padrão de herança autossômico recessivo;
- ✓ Avaliar consanguinidade na família;
- ✓ Avaliar risco para irmãos, novas gestações, parentes em primeiro grau etc.;
- ✓ Diagnóstico precoce é de fundamental importância para avaliar risco genético para futuras gestações.

Direito de uso de imagem concedido à BioMarin.

Williams RE, et al. Poster session presented at: The 12th Annual WORLD Symposium; February-March 2016; San Diego, CA.

Lipofuscinose Ceroide Neuronal Tipo 2 (CLN2)
Tratamento

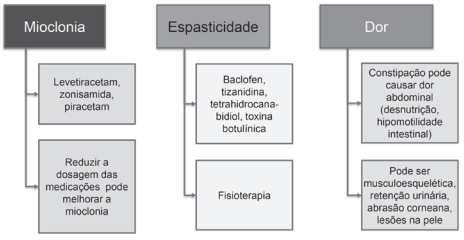
SEGUIMENTO: CONDUTA PADRÃO

O atendimento multidisciplinar promove melhor acompanhamento para crianças com a doença CLN2

Adaptado de: Williams RE, et al. Poster session presented at: The 12th Annual WORLD Symposium; February-March 2016; San Diego, CA.

CONDUTA NA CLN2
TERAPIA SINTOMÁTICA

Sugestão de especialistas em LCNs para tratamento dos sintomas da CLN2



Adaptado de Schulz A. Biochim Biophys Acta. 2013;1832 :1801.

Existem oito ensaios clínicos voltados a NCLs (*clinicaltrials.gov*)

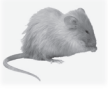
Tipo de CLN	Terapêutica	Mecanismo de ação	Estudos pré-clínicos	Fase do teste
JNCL	Anti-Inflamatorio Micofelato mofetil	Neuro inflamação Produção de AC	Seehafer 2011	Recrutamento
LINCL	Gene terapia AAVrh10CUhCLN2	Engenharia -> células para produzir TPP1 sem mutação	Sondhi 2007 2008 e 20012	Recrutamento
LINCL	Gen terapia AAVrh10CUhCLN2	Engenharia->Células para produzir TPP1 sem mutação	Sondhi 2007 2008 e 20012	Recrutamento
LINCL	TRE (Terapia de Reposição enzimática) BMN 190*	Fonte de TPP1 funcional combinante absorvidas por célula doente	Vuillemenot 2014, 2014	Ativo
LINCL	Gen terapia AAV2CUhCLN2	Engenharia -> células para produzir TPP1 sem mutação	Sandhi,2005 Passini 2006	Ativo
INCL LINCL	Células tronco SNC humano	Celulas tronco como fonte de PPT1 e TPP1 funcionais (~TER)	Tamaki 2009	Completo
INCL	Pequena molécula Cystagon	Limpa lissosoma de material de depósito	Zhang 2001	Completo
INCL LINCL	Células tronco SNC humano	Celulas tronco como fonte de TPP1 funcional (~TER)	Vuillemenot 2014, 2014	Ativo

Geraets RD et al. J Rare Dis. 2016;11:40

*Produto não registrado no Brasil

Modelos animais de CLN2 reproduzem a doença humana

- ✓ Camundongos TPP1-knockout (KO) apresentam tremores, ataxia e perda neuronal;
- ✓ Dachshunds de TPP1-null apresentam déficits visual e cognitivo, ataxia, tremores, crises mioclônicas e atrofia cerebral;
- ✓ Depósito de corpos auto-fluorescentes no SNC em ambos os modelos;
- ✓ Expectativa de vida reduzida: camundongo, 120 dias; dachshunds, 10-11 meses.



Imagens de banco de imagens livre.

Katz ML, et al., Journal of Neuroscience Research 92:1591-1598, 2014; Sleat et al. Neurobiol Dis 2004; Awano et al. Mol Genet Metab 2006

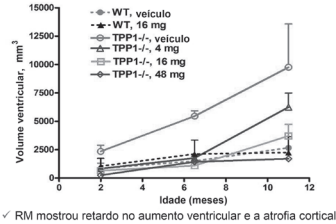
Terapia em cães com TPP1-null (cont.)*

*Produto não registrado no Brasil

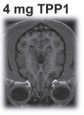
Vídeo cedido pelo Dr Katz ML

Terapia em cães com TPP1-null*

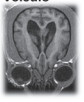
Efeito do tratamento com rhTPP1 no volume ventricular cerebral



4 mg TPP1



Veículo



✓ RM mostrou retardo no aumento ventricular e a atrofia cortical.

*Produto não registrado no Brasil

Adaptado de Katz ML, et al., Journal of Neuroscience Research 92:1591-1598, 2014; Sleat et al. Neurobiol Dis 2004; Awano et al. Mol Genet Metab 2006

TRATAMENTO DA CLN2

✓ **BRINEURA®**

Brineura® (cerliponase alfa)

✓ Aprovada pela FDA, EMA e ANVISA

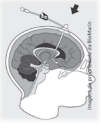
✓ **Reposição Enzimática: Alfacerliponase**

✓ Reposição da enzima: TPP1 humana (**produzida por engenharia genética**);

✓ Alfacerliponase por via intracerebroventricular (ICV);

✓ Dosagem: 300 mg dose fixa;

✓ Frequência: a cada duas semanas.

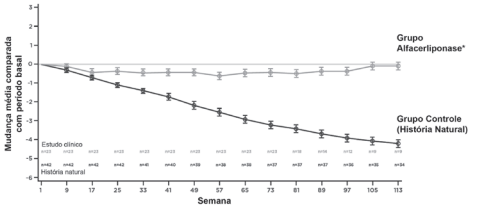


Data on file (FDA): https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/761052Orig1s000TOC.cfm;
http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/004065/human_med_002111.jsp&mid=WC0b01ac058001d124;Vuillemenot BR et al. Mol Genet Metab. 2015;114:281–293; Haltia M. Biochim Biophys Acta. 2006;1762:850–856. Vuillemenot BR et al. Toxicol Appl Pharmacol. 2014;277:49–57

Em humanos, alfacerliponase* estabilizou a evolução clínica da doença CLN2 na maioria dos pacientes em 48 semanas comparados com a história natural da doença

Mudança na média comparada com o período basal na escala de evolução clínica da CLN2

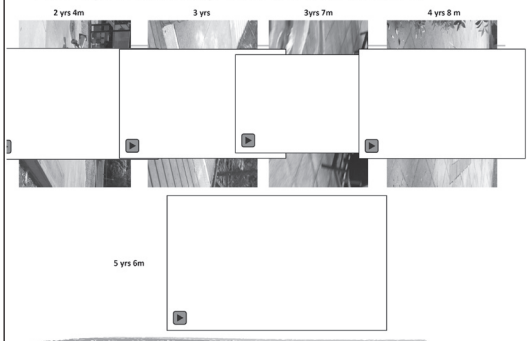
Funções motoras e de linguagem em pacientes tratados (N = 23) *Produto não registrado no Brasil



✓ No estudo 190-201, o declínio médio nos pacientes tratados com alfacerliponase 300mg a cada 2 semanas foi 0,40 pontos em 48 semanas, sendo que a taxa esperada de declínio apontada no grupo de história natural é de perda de 2 pontos a cada 48 semanas.

Data on file (FDA): https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/761052Orig1s000TOC.cfm; Schulz A, et al. International Conference on Neuronal Ceroid Lipofuscinosis. Abstract O-48, 2016.

EVOLUÇÃO DA DOENÇA EM PACIENTE TRATADO



Cedido pelo Dr Nicloa Specchio com permissão do paciente do Hospital Infantil Bambino Gesù

Resumo

- ✓ A doença CLN2:
  - Para crianças com essa patologia neurodegenerativa de progressão rápida, 2 anos até o diagnóstico é muito tempo;
- ✓ Deve-se suspeitar da doença CLN2 em crianças entre 2 a 4 anos de idade com:
  - crises epilépticas não provocadas de início precoce;
  - histórico de atraso precoce de linguagem.
- ✓ Teste enzimático e/ou molecular deve ser usado para diagnosticar a doença CLN2;
- ✓ EEG com FEI de baixa frequência pode ser usado para a suspeita da doença CLN2;
- ✓ O diagnóstico precoce acelera o acesso ao tratamento multidisciplinar específico para a doença CLN2, o que melhorar a qualidade de vida, tanto da criança, como da família.

Schulz A, et al. *Biochim Biophys Acta*. 2013;1832:1801-1806. Mole SE, et al. 2001 Oct 10 [Updated 2013 Aug 1]. In: Pagon RA, et al., eds. GeneReviews. Nickel M, et al. Poster session presented at: The 12th Annual WORLD Symposium; February-March 2016; San Diego, CA. Perez-Poyato MS, et al. *J Child Neurol*. 2012;28:470-478. Fietz M, et al. Poster session presented at: The 12th Annual WORLD Symposium; February-March 2016; San Diego, CA.

ATENÇÃO

1-ATRASO NA FALA -> acompanhar com atenção

2- EPILEPSIA DE INÍCIO RECENTE -> pesquisar etiologia

PARA ALGUMAS DOENÇAS HEREDODEGENERATIVAS EXISTE TRATAMENTO

MAS

O DIAGNÓSTICO PRECOCE É FUNDAMENTAL

Schulz A, et al. *Biochim Biophys Acta*. 2013;1832:1801-1806. Mole SE, et al. 2001 Oct 10 [Updated 2013 Aug 1]. In: Pagon RA, et al., eds. GeneReviews. Nickel M, et al. Poster session presented at: The 12th Annual WORLD Symposium; February-March 2016; San Diego, CA. Perez-Poyato MS, et al. *J Child Neurol*. 2012;28:470-478. Fietz M, et al. Poster session presented at: The 12th Annual WORLD Symposium; February-March 2016; San Diego, CA.

FACILIDADES PARA O DIAGNÓSTICO DA CLN2

REDE DLD BRASIL- Projeto CLN2

Serviço de Genética Médica – Hospital de Clínicas de Porto Alegre
Rua Ramiro Barcelos 22350 - CEP 90035-903- Porto Alegre - RS- SP

Fone gratuito 0800 6438011
Fone (051) 33598010 e Fax (051) 33598010
dld@ufrgs.br

PROJETO "Epilepsia e Genética"

Mendelics

✓**Entre em contato com o Mendelics:**
1- Ligue ou envie um e-mail para o Mendelics
2- Solicite a ficha de critérios de elegibilidade;
3- Depois siga as instruções informadas pelo Mendelics.

✓ +55 11 5096-6001 contato@mendelics.com.br

OBRIGADA

LBE

BIOMARIN

PRESENTATION OF RESEARCH PROJECTS

PRESENTATION OF RESEARCH PROJECTS

GOODBYE TO THE 13TH LASSE