Learning about brain physiology and complexity from the study of the epilepsies

N. Garcia-Cairasco

Laboratório de Neurofisiologia e Neuroetologia Experimental, Departamento de Fisiologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Correspondence to: N. Garcia-Cairasco, Laboratório de Neurofisiologia e Neuroetologia Experimental, Departamento de Fisiologia, FMRP, USP, Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brasil
E-mail: ngcairas@fmrp.usp.br

The brain is a complex system, which produces emergent properties such as those associated with activity-dependent plasticity in processes of learning and memory. Therefore, understanding the integrated structures and functions of the brain is well beyond the scope of either superficial or extremely reductionistic approaches. Although a combination of zoom-in and zoom-out strategies is desirable when the brain is studied, constructing the appropriate interfaces to connect all levels of analysis is one of the most difficult challenges of contemporary neuroscience. Is it possible to build appropriate models of brain function and dysfunctions with computational tools? Among the best-known brain dysfunctions, epilepsies are neurological syndromes that reach a variety of networks, from widespread anatomical brain circuits to local molecular environments. One logical question would be: are those complex brain networks always producing maladaptive emergent properties compatible with epileptogenic substrates? The present review will deal with this question and will try to answer it by illustrating several points from the literature and from our laboratory data, with examples at the behavioral, electrophysiological, cellular and molecular levels. We conclude that, because the brain is a complex system compatible with the production of emergent properties, including plasticity, its functions should be approached using an integrated view. Concepts such as brain networks, graphics theory, neuroinformatics, and e-neuroscience are discussed as new transdisciplinary approaches dealing with the continuous growth of information about brain physiology and its dysfunctions. The epilepsies are discussed as neurobiological models of complex systems displaying maladaptive plasticity.

Key words: Complex systems; Emergence; Epileptology; Neural networks; e-Neuroscience; Neuroinformatics

Learning about brain physiology: the case of the epilepsies

Human and experimental studies of the epilepsies have been considered to be a window to understand the brain. In this respect, it is widely recognized that every protocol designed to test a hypothesis involving hyperexcitable or hypersynchronous brain networks is always contributing *per se* to the increase of knowledge on brain function.

Two historical and excellent proofs of how much we learned over time about brain physiology from epilepsy studies are, first, the classic concept of homunculus defined by the experimental search in human cortical electrical stimulation protocols for areas of representation of the classical sensory and motor functions (1,2). A second and also fascinating protocol is the demonstration by Scoville, Milner and Penfield, of the role of the hippocampus in the formation of memories (3,4) and as an epilepsy-prone region.

These memory studies are related to the experience of HM, a patient who, after a head injury, suffered from temporal lobe epilepsy and because of his refractoriness to phar-

macological treatment was subjected to a bilateral temporal lobotomy and after the surgery suffered a devastating memory loss, with no possibility of acquiring new long-term memories. This clinical description was outstanding, not only because these events called attention to the role of the temporal lobe in the ability to acquire new memories, but also because HM was able to recall information of immediate memory that obviously lasted for some minutes, while he was not distracted. In addition, HM was able to recall very old information, for example from his infancy, which strengthened the view that long-term memories can be stored in cortical areas. Finally, after daily training, amazingly HM was able to improve his motor performance, although not remembering the specific task, a fact that highlights the role of other structures such as the basal ganglia in the expression of the so-called procedural memories (5,6).

Needless to say, current knowledge about the neural substrates associated with contextual and emotional memories adds to those pioneering studies additional levels of complexity that are important factors to be considered not only in epileptic patients but also in patients with neurological and neuropsychiatric co-morbidities. While extremely important, this discussion unfortunately is outside the scope of the present review.

Definitions of complexity, emergence and neural networks

Goldenfeld and Kadanoff (7) state that "...complexity means structures with variations...", and by the same token, "...living forms are complex because of their different working parts, each one formed by variations in the working out of the same genetic coding..." These investigators also contrasted the simplicity of the basic laws of physics with the laws of the world. They claimed that, although every foundation of our worldview obeys laws and everything is simple and eventually expressible in terms of mathematics modeling, what is not simple is the world! They also pondered that outside the physics classroom the world is complex in every corner where we look: mountains, dunes, waves, financial markets, with the biological systems being what they call "...a limiting case of exceptional complexity..." Another "elementary lesson" posed by Goldenfeld and Kadanoff (7) is: "nature can produce complex structures even in simple situations, and can obey simple laws even in complex situations...".

A couple of examples of the difficulty to define the concept of emergence follow. Minsky (8) describes a system in which parts are meaningless, but when working together they form something "greater than the sum of its parts". He then discusses, for example, that intelligence is

an emergent property of the system as it acts and adapts to its environment. Ronald et al. (9) discusses emergence in the field of artificial life in the following terms. "...The description of a phenomenon as emergent is contingent, then, on the existence of an observer; being a visualization constructed in the mind of the observer, emergence can be described as a concept, like beauty or intelligence...". He also considers that the definition of emergence, or of a phenomenon as emergent, needs also the element of surprise, a consequence of the non-obviousness caused sometimes by self-organizing phenomena or by sensitivity to initial conditions (chaos).

Amaral et al. (10) call attention to the increasing focus on the so-called disordered networks because of their potential as models for the interacting networks of complex systems. They talk specifically about small world networks. Briefly, Watts and Strogatz (11) stated that, when connecting nodes with their nearest neighbors, a regular graph is produced that has a high clustering coefficient and high average path length. When edges are randomly rewired (with a probability P), if P = 1, all edges rewire randomly, which means the resultant network is perfectly random (short average path length and clustering coefficient). However, when 0 < P < 1, the resultant graph is a small-world network with high clustering and low path length, a combination of some dense local clustering (regular networks), and some long-range connections (random networks).

A follow-up question was asked by Bassett and Bullmore (12): Why should we think about the brain as a small-world network? They reviewed empirical and theoretical reasons why small worlds present an attractive model for brain network connectivity and also reviewed mathematical methodology and empirical findings in more detail. In summary, Bassett and Bullmore (12) listed what they propose are the reasons that motivate the study of the brain as a small world network: first of all, the brain is a complex network on multiple spatial and time scales; second, the brain supports both segregated and distributed information processing seen in sensorimotor and cognitive processing, localized discretely in specialized regions or large-scale distributed systems; third, the brain probably evolved to maximize efficiency and/or minimize the costs of information processing.

What is interesting for the current review is that there is a growing literature body suggesting that small world network rules fit quite well the normal functional circuits of the brain (13) and even epilepsy-related phenomena (14).

The brain as a complex system: insights from immature and adult brains

In the particular case of the nervous system, Koch and

Laurent (15) highlight that even "simpler" nervous systems have extraordinary complexity reflected in their functions, evolutionary history, structure, and coding schemes for information processing. How to address all these features in an integrated manner is one of the more challenging tasks of current neuroscience research. Because classic and modern techniques allow us to perform a vast number of inquiries into each one of these layers of complexity, Koch and Laurent (15) also emphasize that: "...realistic notions of brain complexity must incorporate, first, the highly nonlinear, nonstationary, and adaptive nature of the neuronal elements themselves and, second, their nonhomogeneous and massive parallel patterns of interconnection whose "weights" can wax and wane across multiple time scales in behaviorally significant ways...".

A clear-cut example of a complex system in which linear expectations are not fulfilled is the one regarding the actions of the commonly known brain GABAergic inhibitory neurotransmission. In fact, when developmentally compared, neonate animals more often express gamma-aminobutyric acid (GABA) depolarization versus GABA hyperpolarization in the adults. These apparently paradoxical effects are, among other factors, related to different chloride ion gating at different ages, which before their description were a matter of conflicting interpretations. Briefly, GABA depolarizing and exciting actions are due to an elevated intracellular Cl^- concentration in immature cells (reviewed in Ref. 16) that are progressively reduced with development.

In the case of neurological disorders, such as the epilepsies, it is obviously intriguing to understand how an adult inhibitory neurotransmitter such as GABA could be potentially pro-epileptogenic in the neonates (17) if we simply consider its depolarizing actions during this period of brain development. Similarly, in a very recent and intriguing study combining the prenatal and neonatal expression of oxytocin with GABA neurotransmission, Tyzio et al. (18) claimed that the depolarizing action of GABA strongly modulated by oxytocin has its major impact as an eventual endogenous protection against hypoxic events with parturition. Furthermore, when inhibition is expected to occur, the apparent paradox further extends at the circuit level, because increases of GABA do not always indicate inhibition. In clinical epileptology research, D'Antuono et al. (19), for example, have shown that in patients with Taylor's type focal cortical dysplasia, epileptiform activity is initiated by a synchronizing mechanism that paradoxically relies on $GABA_A$ receptor activation and is facilitated by the decreased ability of $GABA_B$ receptors to control interneuron GABA release. Furthermore, also in experimental epilepsy, a well-known case is that of the genetically epilepsy-prone rat (GEPR) strain in which increased amounts of GABA neurons and increased messages and protein for its synthetic enzyme glutamic acid descarboxylase (20) are not correlated with enhanced inhibition but rather with lack of inhibition, simply because the enhanced amounts of GABAergic markers are correlated with lack of function. In fact, Faingold et al. (21) demonstrated that at least the GABA system of the inferior colliculus (a triggering area of audiogenic seizures in this strain) is functionally hampered.

Plasticity of normal brain functions as emergent properties of complex systems

When we talk, for example, about sensory systems, such as auditory, visual, olfactory, and others, we accept that the basic processes coupled to the expression of such systems are explained as a combination of very selective stimulus-transduction machinery with mechanisms of transmission of the specific sensory information generally coded by electrochemical features. The concept of sensory perceptions or the cognitive, conscious or cortical levels can also be considered emergent properties of the primary sensory systems. Furthermore, when we talk about the neural development or the mature expression of these systems, their functions need to reflect a synchronous neuroanatomy-neurophysiology coupling. For the reasons above, any sensory system is considered to be a complex system, which is able to produce corresponding emergent properties such as the learning and memory activity-dependent plasticity. Is it not by these mechanisms, obviously added to specific genetic backgrounds and social and psychological environments, that we explain how famous musicians and well-recognized visual artists develop their skills?

The study of learning-memory processes, sensory, motor or cognitive, has been an excellent example of complex systems that are capable of producing associated emergent properties, as already mentioned. Teaching an animal, a child or an adult a given task obviously entrains a set of subsequent cascades from the molecular and cell levels to the system one. As an example, a recent magnetic resonance imaging study by Meister et al. (22) demonstrated this clearly in the evaluation of long-term practice in complex motor tasks by musicians and non-musicians.

Depending on the amount of success of this multilayer activation, the learning process will be accomplished. But, when we investigate these mechanisms, the question is: where do we start? Which one, if anyone alone, is the more adequate, the bottom-up (molecules to systems) or the

top-down (systems to molecules) approach? Is there any conflict in trying both? In the following paragraphs we will try to answer these questions using as models a variety of epileptogenic circuits and their consequent emergent properties.

Epileptogenic circuits as sources of complexity. Epilepsies as emergent properties of these complex systems

If we begin by defining complexity in the studies of epilepsy at different levels, we are at some point forced to organize information in order to increase the understandability of the concepts associated with the data collected. Once we have access to the multitude of data coming from thousands of laboratories worldwide, how much, in fact, should we arrange these data in order to design, probably by network analysis, a coherent explanation of brain function from systems such as *C. elegans* to rodents, non-human primates and human brains?

In order to answer this question we need a fundamental change of paradigm and thus, I propose that epileptogenic networks, a misdirected or non-adaptive result of brain physiology, can be studied as a source of complexity and emergent properties at behavioral, circuit, cell, molecule, and even computational levels.

A recent example of the use of the concepts of complex systems and their emergent properties comes from the studies by Faingold (23) who addressed the ability of brain circuits to change after repeated audiogenic seizures based on the genetic background of GEPRs and in a timely and organized fashion that allows us to look for those altered structures as targets for new pharmacological anticonvulsant and even antiepileptogenic agents.

A recent review by Sutula and Dudek (24) deals with seizure-induced sprouting of the mossy fiber pathway in the dentate gyrus, defined as a nearly universally observed event in experimental models of limbic epilepsy (kindling, status epilepticus) and in the epileptic human hippocampus. Examples come from the experimental kindling model and from temporal lobe seizures in patients. The eventual role of this detected sprouting in the temporal lobe circuitry associated with other plasticity-dependent processes going on in the same brain has been discussed. Sutula and Dudek (24) highlight that an overwhelming majority of sprouted synapses in the inner molecular layer of the dentate gyrus form recurrent excitatory connections, which could be a potential cause of enhanced susceptibility to seizures, but which co-exist with some sprouted axons that form synapses with inhibitory interneurons. As a consequence, recurrent inhibitory circuits are formed,

probably as a compensatory response to prevent seizures. Using an integrated quantitative analysis of the synaptic connections of the sprouted mossy fiber pathway and its functionality as well as complex system analysis, these investigators claimed that functional effects of the recurrent excitatory circuits formed by mossy fiber sprouting after seizures/injury emerge only conditionally and intermittently, as already detected with spontaneous seizures in humans.

After presenting these two examples of the combination of complex systems and emergence, in this section I would like to discuss results from our own experience with these multilayered approaches and try to create a flow of ideas from what appears at first glance to be an apparently disconnected collection of data. Here I would like to challenge our own approach showing how far we are from an actual integrative strategy to study epilepsies and neuroscience in general. But at the same time I would like to highlight that along the path to discovery in epileptology studies we are building up basic concepts and knowledge about brain function. This brings us back to the pioneering studies by Penfield on homunculus and cortical sensorimotor representations and to the studies of Brenda Milner on patient HM and memory, all of which were conducted on epileptic patients. In other words, the fundamental question is how much of brain physiology we can learn from epilepsy studies and, in a more universal view, how much we can learn from other neurological and neuropsychiatric syndromes such as obsessive compulsive disorder, Alzheimer disease, Parkinson disease. Although co-morbidities are common features in several of these patients, it is now more than obvious that the same nervous system was the target of different pathologies that have different time courses. This is indeed a clear-cut proof that the brain is a complex system!

Table 1 presents data as sources of complexity and emergent properties derived in this particular case from experiments on the Wistar audiogenic rat (WAR) strain. The data are arranged as a cross relationship between a given level of complexity (from behavioral to computational levels) and a certain experimental epilepsy model, its relevant features and the associated publications.

To begin with, the WAR strain was developed in our Laboratory as a genetic model of sound-induced reflex epilepsy that in the acute situation models tonic-clonic seizures and in the chronic protocol models temporal lobe epilepsy (25). At the behavioral level it is important to highlight that all acute audiogenic seizures, not only observed in our WAR strain but also in other genetically developed strains, selected in several countries, such as the already mentioned GEPR (26), the French audiogenic

colony (27) and the Chinese audiogenic strain (28), are dependent on the expression of brainstem networks (23). However, once the animal enters a chronic protocol, the so-called audiogenic kindling (28-32), the newly evoked behavioral limbic seizures depend on activation of the recruited cortex, amygdala and hippocampus (29,30,33,34).

Table 1 also shows that, besides detailed behavioral studies with neuroethological tools (31), qualitative EEG alterations have also been reported (33) and more recently EEG quantitative studies have been conducted with the use of wavelet transform analysis (35). Using the latter methodology we began neuropharmacological studies with the WAR strain and demonstrated that phenobarbital, acting mainly on GABAergic neurotransmission, was able to block audiogenic seizures when applied systemically, but not when applied into the substantia nigra reticulata, suggesting a potential genetic alteration in a critical component of an endogenous anticonvulsant system (35).

At the circuit and cellular levels it is clear that audio-genic kindling is followed by positive (neurogenesis) (34) and negative (cell loss) (32) cellular alterations in the absence of Timm-positive mossy fiber sprouting (32,34). Finally, at the molecular level, we are dealing with either cultured hippocampal cell populations (36) or with tissue homogenates from WARs (37), demonstrating endoge-nous and acquired alterations compatible with seizure susceptibility. Quantitative EEG analysis of acute and kindled audiogenic seizure implies the development of computational tools and specific algorithms (38).

The combination of behavioral, circuits, cellular, mo-lecular, and computational environments yields an enor-mous amount of information to deal with, if our main question is integration and computational neuroscience. The progression from a genetically developed brainstem-dependent acute audiogenic seizure to a newly induced forebrain-dependent limbic seizure is at the same time a model of a complex system at all the mentioned levels and of dynamic emergent properties that fit quite well the

Table 1. Sources of complexity and emergent properties in experimental models of epilepsy using Wistar audiogenic rats (WARs).

Level	Model	Relevant features	References
Behavior	Acute audiogenic tonic-clonic seizures	Comparative neuroethological analysis of acute and kindled audiogenic seizures	Garcia-Cairasco et al. (31)
	Kindled audiogenic limbic seizures	Behavioral evidence of limbic regions recruitment	Dutra Moraes et al. (33)
		Genetically developed epileptic inbred strain	Doretto et al. (25)
Circuits	Acute audiogenic tonic-clonic seizures	Inferior colliculus EEG epileptiform activity after acute seizures	Dutra Moraes et al. (33)
	Kindled audiogenic limbic seizures	Cortex, hippocampus and amygdala EEG epileptiform activity after audiogenic kindled seizures	Romcy-Pereira and Garcia-Cairasco (34)
		Systemic but not intranigral phenobarbital blocks audiogenic seizures in WARs	Rossetti et al. (35)
Cellular	Kindled audiogenic limbic seizures	Presence of moderate cell loss (Nissl staining) and BrdU+ dentate granule cell neurogenesis after audiogenic kindled seizures	Galvis-Alonso et al. (32)
		Absence of mossy fiber sprouting in the dentate gyrus after audiogenic kindled seizures	Romcy-Pereira and Garcia-Cairasco (34)
Molecular	Cultured hippocampal cells in naive neonate WARs	Endogenous alterations in hippocampal GABA and glutamate neurotransmission	Mesquita Jr. et al. (36)
	Developmental and adult aspects of seizure susceptibility in WARs	Hippocampal bradykinin B1 and B2 receptors are highly expressed after audiogenic kindled seizures	Pereira et al. (37)
Computational	Sleep studies, acute and kindled audiogenic limbic seizures	Quantitatve EEG measurements (wavelet transform) coupled to behavior evaluations	Romcy-Pereira et al. (38)

proposal made by Faingold cited above (23).

Regarding typical experimental models of temporal lobe epilepsy, Table 2 highlights sources of complexity and emergent properties derived mostly from chemical and electrical manipulations of limbic regions. Deserving some special comments are data from several variations of status epilepticus that we study in our laboratory.

First of all, we applied subconvulsant doses of pilocarpine WAR animals and obtained a stronger status epilepticus compared to Wistar rats (39). In brief, the semiology of the pilocarpine-induced limbic seizures in WARs suggests more severe seizures and a recruitment of brainstem regions, exactly opposite to what we see with recruitment of limbic areas after audiogenic kindling (32-34). In addition, we applied systemic pilocarpine to Wistar rats and evaluated at the cellular level the expression of doublecortin in newly generated granule cells (neurogenesis) (40). We detected clear-cut altered patterns of basal and apical dendrites in those new hippocampal cells. In the latter case, we used computational tools in order to obtain 3-D reconstruction with Neurolucida® (MBF Bioscience, USA). These reconstructed neurons represent structural signatures that, together with altered semiology and electrophysiological signatures, represent multifaceted expressions of complexity and emergence in epileptogenic circuits.

Another variation of the protocol is the intrahippocampal application of pilocarpine in Wistar rats, which induces, with zero mortality, behavioral and EEG evidence of status epilepticus and spontaneous recurrent seizures, the latter an important marker of epileptogenesis and epilepsy. The brains of these animals present Timm-positive mossy fiber sprouting as a clear structural alteration (41).

Following our attempts to selectively stimulate limbic regions instead of obtaining status epilepticus from systemic manipulations, one additional model of temporal lobe seizures is the one described by Nissinen et al. (42). In brief, 20-30 min of electrical amygdala stimulation is able to produce self-sustained status epilepticus after the removal of the stimulation. Using this model in our laboratory, Tilelli et al. (43) demonstrated that two different behavioral patterns of status epilepticus produce two different, NeuN-positive patterns of limbic region lesioning. Finally, we reproduced the amygdala rapid kindling protocol developed by Ebert and Loscher (44) and showed that this protocol is useful because of its rapid evolution after discharges and secondary discharges as well as post-ictal

Table 2. Sources of complexity and emergent properties in experimental models of temporal lobe epilepsy.

Level	Model	Relevant features	References
Behavior	Status epilepticus with subconvulsant doses of systemic pilocarpine in WARs	Combination of homogeneous genetic background (WARs) and limbic seizure experience	Garcia-Cairasco et al. (39)
Circuits	Intrahippocampal pilocarpine-induced status epilepticus in Wistar rats	Spontaneous recurrent seizures, zero mortality, hippocampal and amygdala EEG epileptiform activity with strong hippocampal mossy fiber sprouting	Furtado et al. (41)
	Rapid kindling induced by electrical stimulation of the amygdala	After-discharges, secondary discharges, post-ictal and interictal spikes in hippocampus and amygdala	Foresti et al. (45)
	Self-sustained status epilepticus induced by electrical stimulation of the amygdala	NeuN-positive differential cell damage depending on behavioral subtype of status epilepticus	Tilelli et al. (43)
Cellular	Systemic pilocarpine-induced status epilepticus	Altered patterns of apical and basal dendrites of doublecortin-positive cells	Arisi and Garcia-Cairasco (40)
		Newly generated hippocampal granule cells	Arisi and Garcia-Cairasco (40)
Molecular	Rapid kindling induced by electrical stimulation of the amygdala	Kindling progression in spite of zinc chelation	Foresti et al. (45)
Computational	Systemic pilocarpine-induced status epilepticus	Neurolucida® 3-D reconstruction of doublecortin-positive newly generated hippocampal dentate granule cells	Arisi and Garcia-Cairasco (40)

WARs = Wistar audiogenic rats.

and interictal discharges in both amygdala and hippocampus. What is intriguing regarding these protocols is that we were unable to block the progression of the rapid kindling process despite zinc chelation (45).

The most important issue I would like to discuss, derived from the content of Table 2, is the large amount of information generated again at the behavioral, circuit, cellular, molecular, and computational levels that, if appropriately treated, is clearly another source of complexity and emergent properties. Could the variations in behavior, external markers of epileptogenesis, be explained by EEG, cellular and molecular changes that occur over time with seizures experience? And, what about the behavioral correlates of differential lesioning patterns observed after self-sustained status epilepticus induced by electrical stimulation of the amygdala? What are the functional implications of the dendritic pattern alterations in newly generated granule cells after pilocarpine-induced status epilepticus?

Although the data presented here are mostly related to our own experience, and assuming that the main purpose of this review was not to exclude possible alternative explanations, the major impact of our proposal is to encourage transdisciplinary approaches in brain studies and in epileptology in particular. As a whole, these data also fit the proposal made recently by Sutula and Dudek (24) if we consider their data and ours as specific examples of complex systems that generate dynamic emergent properties. In order to go forward in terms of potential solutions, in the next section I would like to address current strategies that are dealing not only with complexity in regular situations but also in neuropathological conditions such as epilepsy.

Current strategies to deal with complexity and emergence in epileptology, a case for contemporary neuroscience

A whole set of mathematical, statistical and computational tools that is constantly growing is currently available and deals with strategies, such as general algorithms in non-linear dynamics, neural network modeling, small world networks, graph theory and EEG prediction signatures, among others. However, it is not my intention in this review to delve deeply into the details of these methodologies. At the same time I have proposed the need for integration of the experimental designs (either wider or reductionistic), and of the methods of data collection and interpretation in neuroscience and in epileptology in particular. I would like to conclude by leading the discussion to strategies and solutions, mainly with highly complementary, statistical, mathematical and computational algorithms, tools and developments. To this end, Table 3 lists a sample of some of these current method-

ologies. I would like to introduce some examples of these methodologies maintaining the same order of levels of complexity as presented in Tables 1 and 2.

At the behavioral level, I would like to begin by presenting interesting data from the development of Animats by Watts (46) who describes them as computer-simulated animals, or robots that interact with the real world. Animat simulations are considered to be inexpensive and powerful tools to study behavioral mechanisms. Behavior-based artificial intelligence uses Animats capable of autonomous and adaptive activity as conceptual tools in the design of usefully intelligent systems. One particular field where Watts (46) suggests future use, with clear implications for bioethics because of the consequent reduction of experimental animals, is the neuroscience area and epileptology in particular with "...modeling animal movement during human handling and the effects of environmental enrichment on the satisfaction of behavioral needs...".

In an additional contribution, Ohayon et al. (47) developed a network analysis impinging on an external robot with interactive states determined by sensors and motor output, that can mimic either normal (point-fixed) or epileptic (oscillatory with seizure-like motor output) behavior. The dynamics of the interactive network-autonomous agent complex was effective in discriminating normal from pathological conditions, bringing to the field of epileptogenesis modeling an attractive framework that incorporates computational and intelligent tools to distinguish healthy from epileptic networks.

One of the major concerns of the study was, as expressed by the authors: "...to focus the search of network space to identify networks with more complex dynamics. Here we rely on a major available indicator critical to clinical assessment but largely ignored by epilepsy modelers, namely: behavioral states...". Because of the predictions and results from the model, Ohayon et al. (47) stated that: "...these observations turn the question of what causes epilepsy on its head. Instead of asking how epilepsy comes about they compel us to ask how recurrent neural ensembles ever manage to avoid this ubiquitous synchrony in the first place. That is, why are we not all epileptic, all the time?...". This is exactly what we have been asking from the analysis of our experimental epilepsy data (38) over the last two decades: what is more complicated to explain, why more than 98% of the world population is not epileptic or why only less than 2% of the same population have epileptic seizures? At some point this was more evident when we strongly began to experimentally study the so-called endogenous anticonvulsant systems (45,48) and their impact on clinical epileptology (49), in the majority of the mentioned cases with strong quantitative behavioral analysis, which is

quite a real-biology complement of the computational and network approach developed by Ohayon et al. (47).

At the circuit level, further computational approaches have been introduced by Iturria-Medina et al. (13) and Butz et al. (50) (Table 3). Also, Stam (51), for example, applied nonlinear dynamics protocols to the study of epilepsies at the transition from the interictal to the ictal state. At the same time Adeli et al. (52), using wavelet-chaos algorithms, discriminated seizure types using parameters that represent either system complexity (correlation dimension) or system chaocity (largest Lyapunov exponent). When compared, subjects form three groups: healthy, epileptic without seizures (interictal interval) and epileptic during seizures (ictal). The correlation dimension discriminates the three groups for the higher frequency beta and gamma sub-bands, while the largest Lyapunov exponent does so for the lower frequency alpha sub-band.

At the cellular and molecular levels it is possible to find algorithms developed to evaluate massive groups of data such as those currently studied, for example, in proteomic investigations conducted on pilocarpine-induced limbic seizures (53).

Neuroinformatics and e-Neuroscience

The exponential manner in which we are accumulating research results is strongly calling for completely different methods not only of producing the data but also of storing and sharing them (54). For this reason, the so-called Neuroinformatic Projects need to be implemented and

Table 3. Computational strategies to characterize sources of complexity and emergent properties in normal and epileptic brains.

Level	Model/paradigm	Relevant features	References
Behavior	Animats: computer-simulated animals in behavioral research	Robots interacting with the real world or computer simulations	Watts (46)
	Standalone and embodied (autonomous agents) modeling using fully interconnected recurrent neural networks with unit self-feedback	Embodied recurrent network approach discriminates intelligent behavior (computational viability) or pathological conditions (limit cycles or fixed point regions)	Ohayon et al. (47)
Circuits	Anatomical connection probabilities between cortical and subcortical brain gray matter areas estimated from diffusion-weighted magnetic resonance images	Brain modeling as a non-directed weighted graph derived from anatomical connection probability matrix	Iturria-Medina et al. (13)
		Proposed networks with small-world and broad-scale characteristics, with greater local efficiency and smaller global efficiency when compared to random networks	Iturria-Medina et al. (13)
Cellular	Artificial neural network that simulates activity-dependent synaptic reorganization with changing number of neurons, synaptogenesis in recurrent network, recombinations, cell proliferation and apoptosis	Network modeling dynamic remodeling of connectivity patterns by cell proliferation and corresponding neurogenesis, apoptosis and synaptogenesis focusing more on structural and systemic effects of cell proliferation than on computational properties	Butz et al. (50)
Molecular	Systemic pilocarpine-induced status epilepticus	Use of comparative proteomics and projection of signaling networks for simultaneous detection of expression of considerable amounts of proteins validated as potential biomarkers for epilepsy therapies	Liu et al. (53)
Computational	Systemic pilocarpine-induced status epilepticus	Ingenuity pathway analysis® associates proteins detected by proteomics and Western blot/immunohistochemistry with their biological functions	Liu et al. (53)
		Hippocampal expression of a significant group of proteins arranged in networks based on their relationships with biological functions	Liu et al. (53)

spread worldwide. In other words, once we have produced the data we need to store them somewhere in a very easily reachable place so that they can be shared, this being the main goal of neuroinformatics.

An excellent example of data sharing was proposed several years ago, and although its acceptance is not yet widespread, it is growing consistently. Recently, one of its promoters, Giorgio Ascoli (55), devoted new efforts to a strong discussion of the advantages of sharing morphological neuron data, such as 3-D reconstructions using current imaging tools, and of the difficulties or fears alleged by most of those who do not share their data. Specifically, Ascoli (55) states that the success and prestige of molecular biology labs are measured as a proportion of their shared data visibility, while in neuroscience this is still quite a poor routine. However, several projects have increased their support to neuroinformatics initiatives over time. In addition, he states that data sharing also means open-access publishing so that data should become available quickly and freely to the scientific community. A natural consequence, he concludes, is that collaboration, coordination, and computation should yield data, tools and resources needed by neuroscientists. Another important point is related to the difficulties in finding, for example, in the small amount of available neuron-morphology databases, a given type of neuron architecture with given neurotransmitter information with given electrophysiological features. The natural consequence, as he points out, is that the researcher will turn to PubMed and quickly will have his computer screen invaded and in the next second his hard disk with tons of textual information on that matter.

One additional point Ascoli (55) makes is that data sharing is getting easier because currently we have available an enormous amount of freeware software tools for digital neuronal morphology that are used for digital 2-D or 3-D reconstructions. At the same time, lists of these tools that are continuously updated are Neuroscience Database Gateway (56), and Neuron_Morpho (57) from the Krasnow Institute. As a further comment in this regard, Insel et al. (58) strengthen the view that ambitious projects such as transcriptional brains, genomic projects and other goal-directed or large-scale research projects will obligatorily imply transdisciplinary collaboration of scientists mainly in the field of computational neuroscience, where the fundamental issue is that of meaningful data sharing.

An initial effort in this field in our Laboratory has been made in the context of research directed at the 3-D reconstruction of newly generated doublecortin-positive dentate granule cells after status epilepticus induced by systemic application of pilocarpine. The final step, besides having the whole set of experiments published by Arisi and Garcia-Cairasco (40), was to upload those 3-D reconstructions in the NeuroMorpho Project at the Krasnow Institute. At the electrophysiological level, we have also developed tools to do EEG quantitative analysis from sleep and epilepsy data (38) and the algorithms are also available as supplementary material in the electronic version of that article. With these efforts we are slowly but steadily contributing to this new phase of contemporary neuroscience where data sharing is the fundamental cornerstone.

Conclusions and Perspectives

There is no doubt that we can learn about brain physiology from epilepsy studies and, by analogy, from other neurological and neuropsychiatric disorders, recognizing than integrated, transdisciplinary research strategies are better than isolated ones.

The use of computational models in neuropsychiatry has been reviewed recently by Rolls et al. (59), particularly as an attempt to study diminished stability and noisy neurodynamic behavior of prefrontal cortex networks in schizophrenia. In addition, our laboratory has extended current views in epileptology with what we called a "puzzling challenge" in contemporary neuroscience (60), meaning that the critical addition of multiple approaches will bring more appropriate ways to convey real data with their modeling algorithms. However, no matter which specific analysis we are dealing with in the neuroscience and in this case in epileptology studies, at the behavioral, circuit, cellular, molecular, and event computational levels, the end point will always be the human interface, who decides, wisely or not, how to approach, either reductionistically or not, in an integrated manner or not, with transdisciplinary strategies or not, how to conduct the experiments and the research programs. The data collection methods as well as the new technologies are more and more widely available. Progress on multiple fronts will occur as more groups engage in transdisciplinary collaboration.

Acknowledgments

References

1. Penfield W, Boldrev E. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. *Brain* 1937; 60: 389-443.

2. Schott GD. Penfield's homunculus: a note on cerebral cartography. *J Neurol Neurosurg Psychiatry* 1993; 56: 329-333.

3. Milner B, Penfield W. The effect of hippocampal lesions on recent memory. *Trans Am Neurol Assoc* 1955; 42-48.

4. Scoville WB, Milner B. Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry* 1957; 20: 11-21.

5. Milner B. The medial temporal-lobe amnesic syndrome. *Psychiatr Clin North Am* 2005; 28: 599-611, 609.

6. Squire LR, Kandel E. *Memory: From brain to molecules*. New York: Scientific American Library; 1999.

7. Goldenfeld N, Kadanoff LP. Simple lessons from complexity. *Science* 1999; 284: 87-89.

8. Minsky M. *The society of mind*. New York: Simon and Schuster, Inc.; 1986.

9. Ronald EM, Sipper M, Capcarrere MS. Design, observation, surprise! A test of emergence. *Artif Life* 1999; 5: 225-239.

10. Amaral LA, Scala A, Barthelemy M, Stanley HE. Classes of small-world networks. *Proc Natl Acad Sci U S A* 2000; 97: 11149-11152.

11. Watts DJ, Strogatz SH. Collective dynamics of 'small-world' networks. *Nature* 1998; 393: 440-442.

12. Bassett DS, Bullmore E. Small-world brain networks. *Neuroscientist* 2006; 12: 512-523.

13. Iturria-Medina Y, Sotero RC, Canales-Rodriguez EJ, Aleman-Gomez Y, Melie-Garcia L. Studying the human brain anatomical network via diffusion-weighted MRI and Graph Theory. *Neuroimage* 2008; 40: 1064-1076.

14. Ponten SC, Bartolomei F, Stam CJ. Small-world networks and epilepsy: graph theoretical analysis of intracerebrally recorded mesial temporal lobe seizures. *Clin Neurophysiol* 2007; 118: 918-927.

15. Koch C, Laurent G. Complexity and the nervous system. *Science* 1999; 284: 96-98.

16. Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev* 2007; 87: 1215-1284.

17. Veliskova J, Claudio OI, Galanopoulou AS, Lado FA, Ravizza T, Velisek L, et al. Seizures in the developing brain. *Epilepsia* 2004; 45 (Suppl 8): 6-12.

18. Tyzio R, Cossart R, Khalilov I, Minlebaev M, Hubner CA, Represa A, et al. Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science* 2006; 314: 1788-1792.

19. D'Antuono M, Louvel J, Kohling R, Mattia D, Bernasconi A, Olivier A, et al. GABAA receptor-dependent synchronization leads to ictogenesis in the human dysplastic cortex. *Brain* 2004; 127: 1626-1640.

20. Roberts RC, Ribak CE, Oertel WH. Increased numbers of GABAergic neurons occur in the inferior colliculus of an audiogenic model of genetic epilepsy. *Brain Res* 1985; 361: 324-338.

21. Faingold CL, Gehlbach G, Travis MA, Caspary DM. Inferior colliculus neuronal response abnormalities in genetically epilepsy-prone rats: evidence for a deficit of inhibition. *Life Sci* 1986; 39: 869-878.

22. Meister I, Krings T, Foltys H, Boroojerdi B, Muller M, Topper R, et al. Effects of long-term practice and task complexity in musicians and nonmusicians performing simple and complex motor tasks: implications for cortical motor organization. *Hum Brain Mapp* 2005; 25: 345-352.

23. Faingold CL. Emergent properties of CNS neuronal networks as targets for pharmacology: application to anticonvulsant drug action. *Prog Neurobiol* 2004; 72: 55-85.

24. Sutula TP, Dudek FE. Unmasking recurrent excitation generated by mossy fiber sprouting in the epileptic dentate gyrus: an emergent property of a complex system. *Prog Brain Res* 2007; 163: 541-563.

25. Doretto MC, Fonseca CG, Lobo RB, Terra VC, Oliveira JA, Garcia-Cairasco N. Quantitative study of the response to genetic selection of the Wistar audiogenic rat strain (WAR). *Behav Genet* 2003; 33: 33-42.

26. Jobe PC, Picchioni AL, Chin L. Role of brain norepinephrine in audiogenic seizure in the rat. *J Pharmacol Exp Ther* 1973; 184: 1-10.

27. Marescaux C, Vergnes M, Kiesmann M, Depaulis A, Micheletti G, Warter JM. Kindling of audiogenic seizures in Wistar rats: an EEG study. *Exp Neurol* 1987; 97: 160-168.

28. Li SY, Xu DS, Jia HT. AGS-induced expression of Narp is concomitant with expression of AMPA receptor subunits GluR1 and GluR2 in hippocampus but not inferior colliculus of P77PMC rats. *Neurobiol Dis* 2003; 14: 328-335.

29. Vergnes M, Kiesmann M, Marescaux C, Depaulis A, Micheletti G, Warter JM. Kindling of audiogenic seizures in the rat. *Int J Neurosci* 1987; 36: 167-176.

30. Naritoku DK, Mecozzi LB, Aiello MT, Faingold CL. Repetition of audiogenic seizures in genetically epilepsy-prone rats induces cortical epileptiform activity and additional seizure behaviors. *Exp Neurol* 1992; 115: 317-324.

31. Garcia-Cairasco N, Wakamatsu H, Oliveira JA, Gomes EL, Del Bel EA, Mello LE. Neuroethological and morphological (Neo-Timm staining) correlates of limbic recruitment during the development of audiogenic kindling in seizure susceptible Wistar rats. *Epilepsy Res* 1996; 26: 177-192.

32. Galvis-Alonso OY, Cortes De Oliveira JA, Garcia-Cairasco N. Limbic epileptogenicity, cell loss and axonal reorganization induced by audiogenic and amygdala kindling in Wistar audiogenic rats (WAR strain). *Neuroscience* 2004; 125: 787-802.

33. Dutra Moraes MF, Galvis-Alonso OY, Garcia-Cairasco N. Audiogenic kindling in the Wistar rat: a potential model for recruitment of limbic structures. *Epilepsy Res* 2000; 39: 251-259.

34. Romcy-Pereira RN, Garcia-Cairasco N. Hippocampal cell proliferation and epileptogenesis after audiogenic kindling are not accompanied by mossy fiber sprouting or Fluoro-Jade staining. *Neuroscience* 2003; 119: 533-546.

35. Rossetti F, Rodrigues MC, de Oliveira JA, Garcia-Cairasco N. EEG wavelet analyses of the striatum-substantia nigra pars reticulata-superior colliculus circuitry: audiogenic seizures and anticonvulsant drug administration in Wistar audiogenic rats (War strain). *Epilepsy Res* 2006; 72: 192-208.

36. Mesquita F Jr, Aguiar JF, Oliveira JA, Garcia-Cairasco N,

Varanda WA. Electrophysiological properties of cultured hippocampal neurons from Wistar Audiogenic Rats. *Brain Res Bull* 2005; 65: 177-183.

37. Pereira MG, Gitai DL, Paco-Larson ML, Pesquero JB, Garcia-Cairasco N, Costa-Neto CM. Modulation of B1 and B2 kinin receptors expression levels in the hippocampus of rats after audiogenic kindling and with limbic recruitment, a model of temporal lobe epilepsy. *Int Immunopharmacol* 2008; 8: 200-205.

38. Romcy-Pereira RN, de Araujo DB, Leite JP, Garcia-Cairasco N. A semi-automated algorithm for studying neuronal oscillatory patterns: a wavelet-based time frequency and coherence analysis. *J Neurosci Methods* 2008; 167: 384-392.

39. Garcia-Cairasco N, Rossetti F, Oliveira JA, Furtado MA. Neuroethological study of status epilepticus induced by systemic pilocarpine in Wistar audiogenic rats (WAR strain). *Epilepsy Behav* 2004; 5: 455-463.

40. Arisi GM, Garcia-Cairasco N. Doublecortin-positive newly born granule cells of hippocampus have abnormal apical dendritic morphology in the pilocarpine model of temporal lobe epilepsy. *Brain Res* 2007; 1165: 126-134.

41. Furtado Mde A, Braga GK, Oliveira JA, Del Vecchio F, Garcia-Cairasco N. Behavioral, morphologic, and electroencephalographic evaluation of seizures induced by intrahippocampal microinjection of pilocarpine. *Epilepsia* 2002; 43 (Suppl 5): 37-39.

42. Nissinen J, Halonen T, Koivisto E, Pitkanen A. A new model of chronic temporal lobe epilepsy induced by electrical stimulation of the amygdala in rat. *Epilepsy Res* 2000; 38: 177-205.

43. Tilelli CQ, Del Vecchio F, Fernandes A, Garcia-Cairasco N. Different types of status epilepticus lead to different levels of brain damage in rats. *Epilepsy Behav* 2005; 7: 401-410.

44. Ebert U, Loscher W. Differences in mossy fibre sprouting during conventional and rapid amygdala kindling of the rat. *Neurosci Lett* 1995; 190: 199-202.

45. Foresti ML, Arisi GM, Fernandes A, Tilelli CQ, Garcia-Cairasco N. Chelatable zinc modulates excitability and seizure duration in the amygdala rapid kindling model. *Epilepsy Res* 2008; 79: 166-172.

46. Watts JM. Animats: computer-simulated animals in behavioral research. *J Anim Sci* 1998; 76: 2596-2604.

47. Ohayon EL, Kalitzin S, Suffczynski P, Jin FY, Tsang PW, Borrett DS, et al. Charting epilepsy by searching for intelligence in network space with the help of evolving autonomous agents. *J Physiol* 2004; 98: 507-529.

48. Rodrigues MC, Rossetti F, Foresti ML, Arisi GM, Furtado MA, Dal-Col ML, et al. Correlation between shaking behaviors and seizure severity in five animal models of convulsive seizures. *Epilepsy Behav* 2005; 6: 328-336.

49. Cleto Dal-Col ML, Bertti P, Terra-Bustamante VC, Velasco TR, Araujo Rodrigues MC, Wichert-Ana L, et al. Is dystonic posturing during temporal lobe epileptic seizures the expression of an endogenous anticonvulsant system? *Epilepsy Behav* 2008; 12: 39-48.

50. Butz M, Lehmann K, Dammasch IE, Teuchert-Noodt G. A theoretical network model to analyse neurogenesis and synaptogenesis in the dentate gyrus. *Neural Netw* 2006; 19: 1490-1505.

51. Stam CJ. Nonlinear dynamical analysis of EEG and MEG: review of an emerging field. *Clin Neurophysiol* 2005; 116: 2266-2301.

52. Adeli H, Ghosh-Dastidar S, Dadmehr N. A wavelet-chaos methodology for analysis of EEGs and EEG subbands to detect seizure and epilepsy. *IEEE Trans Biomed Eng* 2007; 54: 205-211.

53. Liu XY, Yang JL, Chen LJ, Zhang Y, Yang ML, Wu YY, et al. Comparative proteomics and correlated signaling network of rat hippocampus in the pilocarpine model of temporal lobe epilepsy. *Proteomics* 2008; 8: 582-603.

54. Koslow SH, Hirsch MD. Celebrating a decade of neuroscience databases: looking to the future of high-throughput data analysis, data integration, and discovery neuroscience. *Neuroinformatics* 2004; 2: 267-270.

55. Ascoli GA. Mobilizing the base of neuroscience data: the case of neuronal morphologies. *Nat Rev Neurosci* 2006; 7: 318-324.

56. Neuroscience Database Gateway [Online]. *Society for Neuroscience (SfN)* http://ndg.sfn.org/; 2008.

57. The NeuroMorpho.org Inventory [Online]. *Krasnow Institute for Advanced Study* http://neuromorpho.org/neuroMorpho/index.jsp; 2008.

58. Insel TR, Volkow ND, Li TK, Battey JF Jr, Landis SC. Neuroscience networks: data-sharing in an information age. *PLoS Biol* 2003; 1: E17.

59. Rolls ET, Loh M, Deco G, Winterer G. Computational models of schizophrenia and dopamine modulation in the prefrontal cortex. *Nat Rev Neurosci* 2008; 9: 696-709.

60. Garcia-Cairasco N. Puzzling challenges in contemporary neuroscience: insights from complexity and emergence in epileptogenic circuits. *Epilepsy Behav* 2009; 14 (Suppl 1): 54-63.