Commonalities in epileptogenic processes from different acute brain insults. Does it translate?

Pavel Klein[1], Raymond Dingledine[2], Eleonora Aronica[3], Christophe Bernard[4], Ingmar Blümcke[5], Detlev Boison[6], Martin J. Brodie[7], Amy R. Brooks-Kayal[8], Jerome Engel, Jr.[9], Patrick A. Forcelli[10], Lawrence J. Hirsch[11], Rafal M. Kaminski[12], Henrik Klitgaard[12], Katja Kobow[5], Daniel H. Lowenstein[13], Phillip L. Pearl[14], Asla Pitkänen[15], Noora Puhakka[15], Michael A. Rogawski[16], Dieter Schmidt[17], Matti Sillanpää[18], Robert S. Sloviter[19], Christian Steinhäuser[20], Annamaria Vezzani[21], Matthew C. Walker[22], and Wolfgang Löscher[23]

1 Mid-Atlantic Epilepsy and Sleep Center, Bethesda, MD, USA
2 Department of Pharmacology, Emory University, Atlanta, GA, USA 30322
3 Department of (Neuro)Pathology, Academic Medisch Centrum (AMC) and Swammerdam Institute for Life Sciences, Center for Neuroscience, University of Amsterdam, and Stichting Epilepsie Instellingen Nederland (SEIN), The Netherlands
4 Aix Marseille Université, Inserm, INS UMR S 1106, Marseille, France
5 Department of Neuropathology, University Hospital Erlangen, Erlangen, Germany
6 Robert Stone Dow Neurobiology Laboratories, Legacy Research Institute, Portland, OR, USA
7 Epilepsy Unit, West Glasgow ACH-Yorkhill, Glasgow G3 8SJ, Scotland, UK
8 Division of Neurology, Departments of Pediatrics and Neurology; University of Colorado School of Medicine; Aurora, CO USA; Children's Hospital Colorado; Aurora, CO USA; Neuroscience Program; University of Colorado; Anschutz Medical Campus; Aurora, CO USA
9 Departments of Neurology, Neurobiology, and Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, The Brain Research Institute, University of California, Los Angeles, CA, USA
10 Department of Pharmacology, Georgetown University, Washington, DC, USA
11 Department of Neurology, Yale University, New Haven, CT 06831, USA
12 UCB Pharma, Braine-l'Alleud, Belgium
13 Department of Neurology, University of California, San Francisco, CA 94143, USA
14 Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA
15 Department of Neurobiology, AI Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland
16 Department of Neurology, University of California, Davis, Sacramento, CA 95817, USA
17 Epilepsy Research Group, 14163 Berlin, Germany
18 Departments of Child Neurology and General Practice, University of Turku and Turku University Hospital, Turku, Finland
19 Department of Neurobiology, Morehouse School of Medicine, Atlanta, GA, USA
20 Institute of Cellular Neurosciences, Medical Faculty, University of Bonn, Germany
21 Department of Neuroscience, IRCCS-"Mario Negri" Institute for Pharmacological Research, Milan, Italy
22 Department of Clinical & Experimental Epilepsy, UCL Institute of Neurology, London, United Kingdom
23 Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine, Hannover, and Center for Systems Neuroscience, Hannover, Germany

Correspondence to: Dr. W. Löscher, Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany; E-mail: **wolfgang.loescher@tiho-hannover.de**

Abstract

The most common forms of acquired epilepsies arise following acute brain insults such as traumatic brain injury, stroke, or CNS infections. Treatment is effective for only 60-70% of patients and remains symptomatic despite decades of effort to develop epilepsy prevention therapies. Recent preclinical efforts are focused on likely primary drivers of epileptogenesis, namely inflammation, neuron loss, plasticity and circuit reorganization. This review suggests a path to identify neuronal and molecular targets for clinical testing of specific hypotheses about epileptogenesis and its prevention or modification. Acquired human epilepsies with different etiologies share some features with animal models. We identify these commonalities and discuss their relevance to development of successful epilepsy prevention or disease modification strategies. Risk factors for developing epilepsy that appear common to multiple acute injury etiologies include intracranial bleeding, disruption of the blood-brain barrier, more severe injury and early seizures within a week of injury. In diverse human epilepsies and animal models, seizures appear to propagate within a limbic or thalamocortical/cortico-cortical network. Common histopathology features of epilepsy of diverse and mostly focal origin are microglial activation and astrogliosis, heterotopic neurons in the white matter, loss of neurons, and the presence of inflammatory cellular infiltrates. Astrocytes exhibit smaller K^+ conductances and lose gap junction coupling in many animal models as well as in sclerotic hippocampi from temporal lobe epilepsy patients. There is increasing evidence that epilepsy can be prevented or aborted in preclinical animal models of acquired epilepsy by interfering with processes that appear common to multiple acute injury etiologies, e.g., in post-status epilepticus models of focal epilepsy by transient treatment with a trkB/PLCγ1 inhibitor, isoflurane or HMGB1 antibodies and by topical administration of adenosine, in the cortical fluid percussion injury model by focal cooling, and in the albumin post-traumatic epilepsy model by losartan. Preclinical studies further highlight the roles of mTOR1 pathways, JAK-STAT3, IL-1R/TLR4 signaling and other inflammatory pathways in the genesis or modulation of epilepsy after brain injury. The wealth of commonalities, diversity of molecular targets identified preclinically, and likely multidimensional nature of epileptogenesis argue for a combinatorial strategy in prevention therapy. Going forward, the identification of impending epilepsy biomarkers to allow better patient selection, together with better alignment with multi-site preclinical trials in animal models, should guide the clinical testing of new hypotheses for epileptogenesis and its prevention.

Key points

- Epileptogenesis after common acute brain insults shares enough features to consider it as one broad category

- We identify clinical and preclinical commonalities in epileptogenic processes and their relevance to successful epilepsy prevention strategies

- Status epilepticus (SE) model findings should be corroborated in trauma and stroke models to see whether in future SE data can be used to support human studies

- Preclinical evidence for several recently-identified preventive therapeutic strategies can be followed in human studies

Introduction and clinical context

Approximately 20-60% of all epilepsy is caused by acute CNS insults[1]. In the U.S., traumatic brain injury (TBI) causes approximately 6% of all epilepsies, cerebrovascular accident (CVA) 11%, infections 4%, and new onset cryptogenic status epilepticus (SE) <1%[2].

The ability to prevent epilepsy after brain injury or reduce its severity is one of the great unmet needs in neurology[3]. A latent period of days to years often exists between the acute insult and the onset of clinically obvious epilepsy[4] (Fig. 1). This post-injury interval offers an opportunity to intervene with treatment to prevent or delay epilepsy. No preventive treatment exists, yet few preventive studies have been conducted during the last 25 years despite the availability of a variety of drugs marketed for non-epilepsy indications that may have antiepileptogenic or disease-modifying effects in animal models.[3,5,6,7] One reason for this is that -in the absence of prospective invasive EEG data- the incidence of epilepsy after acute epileptogenic insults appears relatively low (e.g., 20-50% after moderate to severe TBI), and the clinical diagnosis of spontaneous ("late") seizures can take years, making clinical trials logistically challenging. Furthermore, there is uncertainty about whether the results of animal studies can be extrapolated to humans. The most commonly used animal models of epileptogenesis are post-SE models of temporal lobe epilepsy (TLE) and kindling models. The validity of applying results from these models to human epilepsy developing after TBI, CVA or infections has not been established.

In some implementations of fluid percussion injury (FPI; a model of TBI) with invasive-EEG monitoring, only 20-50% of the animals appear to develop epilepsy and their epileptic seizures are infrequent.[8,9] However, optimization of the FPI model to maximize incidence and speed of epileptogenesis, and EEG montage to detect focal perilesional neocortical seizures, has resulted in a post-traumatic epilepsy (PTE) model that produces rapid epileptogenesis (>90% incidence of spontaneous seizures within 1 month of brain injury) and frequent seizures, including focal neocortical seizures that do not spread to the

hippocampus, and this model has been successfully deployed in anti-epileptogenesis studies.[10,11]

Clinical evidence points to common elements among the acute brain injuries that lead to human epilepsy (Fig. 1), which may provide insights into common mechanisms of epileptogenesis. (Box 1 and Supplementary Text 1). Although only a small proportion of patients with TBI, CVA and infections develop epilepsy, certain subgroups of patients have a higher risk. Risk factors (Table 1) may provide insights into common mechanisms of epileptogenesis. The highest risk factors for epilepsy after acute injury include extravasation of blood due to breach of blood vessels (TBI with intracranial hemorrhage, CVA due to cerebral hemorrhage or ischemic CVA with hemorrhagic conversion), severe injury, disruption of blood-brain barrier (BBB) with cerebral edema (encephalitis, febrile SE), early seizures occurring within one week after injury, and involvement of frontal or temporal lobes (Table 1). All but locus of injury are associated with an inflammatory response; other converging mechanisms by which these risk factors may promote epilepsy are discussed in Supplementary Text 1. The focal epilepsies resulting from such brain insults, e.g., TLE with hippocampal alterations or neocortical epilepsy, which can also involve hippocampal abnormalities, are often similar and reinforce the notion of commonalities in epileptogenic processes.

The purpose of this review is to examine the evidence for possible commonalities in epileptogenic processes among different causes of acute injury in humans, and among different animal models of acquired epileptogenesis. We attempt to answer the question whether evidence from diverse animal models can be applied across the different common causes of human epilepsy after acute injury, and be utilized in translational studies of epilepsy prevention.

Clinical evidence for commonalities in focal epilepsies resulting from different acute injuries in adults and children is described in more detail in Supplementary Text 1.

Acute brain injury EEG findings and how they relate to later epilepsy

The similarities in acute EEG findings in a variety of acute brain injuries are much more striking than the differences. Nonconvulsive seizures are seen in 13-25% overall, and are increasingly seen with more cortical involvement, more intraparenchymal blood, acute clinical seizures, and the presence of coma.[12,13] Similarly, spreading depolarizations (a form of peri-injury spreading depression seen on intracranial EEG) are common in a variety of acute brain injuries, including subarachnoid hemorrhage, TBI, spontaneous intraparenchymal hemorrhage, and malignant ischemic stroke.[14] Acute nonconvulsive seizures, periodic discharges and spreading depolarizations have been independently correlated with adverse physiological effects and worse neurological outcomes in numerous studies.[15]

Only a few investigations have looked at the later development of epilepsy based on acute EEG findings, but they seem to have predictive value as well. For example, a study of children undergoing continuous EEG monitoring in the pediatric ICU showed that 47% of those with acute symptomatic electrographic SE went on to develop epilepsy, versus 11% in those without this EEG finding.[16] Electrographic SE had an odds ratio of 13.3 for development of epilepsy; it was also associated with worse functional outcome and quality of life. In a study of >100 survivors who had lateralized periodic discharges (LPDs) or nonconvulsive seizures (NCSzs), new development of epilepsy was seen in 17% of those with LPDs only, 38% with NCSzs only, and 48% with both LPDs and NCSzs.[17] Thus, patients with these acute EEG findings may be an appropriate cohort for epilepsy-prevention trials.

In critically ill patients, invasive EEG reveals seizures and PDs in a higher percentage of patients than can be seen on scalp EEG, some of which appear to be causing metabolic distress.[18,19] Whether these findings will predict later epilepsy, or whether detection of acute high-frequency oscillations (HFOs) with this type of recording will be as predictive as they are in animal models[20], remains to be determined.

Thus, there appear to be commonalities in the acute EEG findings and in their prognostic significance, regardless of the specific type of acute brain injury. However, there has been minimal work on whether serial scalp EEGs can detect those patients that are in the process of developing epilepsy, or whether the specific type of acute brain injury matters once a given pattern is seen.

Genetic aspects in patients

There is little doubt that genetic factors have a role in the likelihood of developing epilepsy after acquired acute brain insults. The study of the genetic basis of familial epilepsies with Mendelian inheritance and the epileptic encephalopathies has clearly established the role of genes expressing ion channels, components of synaptic machinery, and other cellular determinants of network excitability.[21,22] Some of these same genetic variants (e.g., DEPDC5, LGI1, PCDH19, SCN1A, GRIN2A, KCNQ2, GABRG2, and others) are weakly associated with common forms of non-acquired epilepsy where a genetic basis is suspected given there is no identifiable insult or lesion.[23] Thus, as suggested decades ago[24], it is highly likely that variants in these and other genes in normal humans influence the likelihood of developing epilepsy after acute brain injury. This concept is supported by the observation that the genetic background of rodents can be a major determinant in the development of chronic, recurrent seizures following a variety of brain insults[25]. The observation that coexpression of two different genes with epilepsy-causing mutations can mask the severity of epilepsy caused by each acting individually[26] likely contributes to the variable penetrance of epilepsy risk genes. However, it is not yet known whether any of the mechanisms of network dysfunction associated with the myriad of genetic epilepsies overlap with the underlying molecular basis of the acquired epilepsies.

Few clinical or epidemiological studies of epilepsy following brain injury have investigated the role of genetics. Jennett reported that a family history of epilepsy was more

common in young patients with posttraumatic epilepsy[27], but other studies showed no association in patients of any age.[28,29,30] Ottman et al. found no increased family history of epilepsy in patients who developed chronic seizures after stroke or meningitis/encephalitis. In the most definitive study of TBI to date, Christensen and colleagues[31] followed more than 1.6 million Danish children and adults for up to 30 years after TBI, and found that patients with a family history of epilepsy had a notably higher risk of epilepsy after mild (Relative Risk 5.75) and severe (10.1) TBI. In epilepsy developing after SE, twin studies have suggested a clear genetic influence in both febrile and afebrile SE[32,33], but no large-scale studies in the general population have been completed to date.

With regard to the identification of *specific* genetic variants associated with the development of epilepsy following brain injury in humans, most findings come from studies of TBI. A recent review by Bennett et al.[34] summarizes the results of 31 separate studies describing polymorphisms in 24 genes, including genes involved in the inflammatory response (e.g. pro- and anti-inflammatory cytokines such as tumor necrosis factor(TNF)-α and interleukin(IL)-6), neuronal plasticity (e.g. brain-derived neurotrophic factor, BDNF), and cognitive function (e.g. catechol-O-methyl transferase). Some of these genes are of particular interest given the roles of inflammatory factors and growth factors in epileptogenesis.[35,36] Other reports have implicated glutamic acid decarboxylase[37], glutamate transport genes[38], and the adenosine A1 receptor[39]. Only two studies have reported genetic associations with PSE; one identified a polymorphism in a TNF receptor, CD4[40], and the other in mitochondrial aldehyde dehydrogenase 2[41]. Much more work is required to verify these findings as well as their biological relevance.

Common neuropathology findings in focal human epilepsies

The neuropathology in 9,523 patients with focal epilepsy and epilepsy surgery in 36 European centers was recently reviewed (European Epilepsy Brain Bank; for details see Blümcke et

al.[42]). 36 histopathology diagnoses were described, classified into seven categories. Hippocampal sclerosis, brain tumors, and malformations of cortical development (MCD) were most common, accounting for 78.8% of the series. 72% of lesions were located in the temporal lobe, compared to 13% in frontal and 9% in multiple lobes.

The following structural commonalities were identified:

1) Reactive astrogliosis is always visible in epilepsy surgery specimens.[43] In the neocortex of patients with epilepsy, astrocytes are typically activated at the BBB or blood-CSF-barrier (Fig. 2A). In subcortical white matter, fibrillary (or spider) gliosis can be seen. It is difficult to microscopically identify single astrocytes in this condition as they are covered and hidden by a dense meshwork of spider-like ramifying processes (Fig. 2B). This pattern is the likely cause for neuroimaging changes in white matter of patients with TLE.[44] Astrocytes may play a significant role in epileptogenesis (see separate section below).

2) Excess of heterotopic neurons in white matter can be seen in many epilepsy-surgery specimens, such as hippocampal sclerosis or MCD (Fig. 3). This is referred to as "mild malformation of cortical development" when detected only as microscopic abnormality [45,46] Heterotopic neurons are rare in other brain diseases, but consistently reported in epilepsy.[45,48,49] No pathomechanistic hypotheses have been developed yet from this observation.

3) White matter angiopathy is also common[50], seemingly unrelated to a specific disease category, but associated with early seizure onset and long-seizure duration.

4) Inflammatory cellular infiltrates are frequently found. Lymphocytic infiltration can be provoked during peri-surgical manipulation of brain tissue. Intracranial neurophysiology techniques applied during pre-surgical evaluation can also induce a mixed lymphocytic-microglial signature.

A common feature of these histopathology findings is anatomical localization in the white matter which, in epilepsy, is an under-studied anatomical compartment. The

epileptogenic lesion is connected via axonal projections to its target regions, together referred to as the *seizure network*. In focal epilepsy, synchronized activation within the seizure network produces the clinical semiology. As yet, we have neither basic nor clinical evidence for seizures arising directly from white matter.

In addition to these histopathology findings, epigenetic alterations may be involved in epileptogenesis.[51] Electrical membrane activity transmits directly into the cell nucleus, thereby driving and adjusting a neuron's response, including molecular memories that underlie long-lasting response to the environment, i.e. the molecular memory of a neuron.[52] Epigenetic histone code modification and long-lasting DNA methylation are a hallmark of molecular memory.[53] Increased DNA methylation patterns occur in various animal models of epilepsy and in drug-resistant human epileptic brain tissue.[51,54-57] It can be hypothesized that sustained epileptic activity alters the molecular memory of a neuron, promoting the development of a chronic epileptic state, which can be termed "epileptogenic memory".

Commonalities in routes of seizure propagation from different epileptic foci

Commonalities in the pathways of seizure propagation are likely a function of the underlying network architecture - with circuits permissive to re-entrant activity being particularly vulnerable to ictogenesis.[58] In some models of epilepsy (e.g., amygdala kindling, intra-hippocampal kainate injection) primary injury is localized, whereas in others (e.g., SE) injury is diffuse, yet generally, the seizure phenotype is the same.[59] Injuries after TBI, hypoxia/ischemia, or infection may likewise be focal or diffuse, and similarly engage two primary networks when propagating beyond the initial focus: (1) the limbic seizure network seen in TLE, and (2) a thalamocortical network similar to that seen in absence epilepsies, characterized by bilateral spike-and-wave discharges (SWDs) and behavioral arrest. Simply put, because the routes of seizure propagation are constrained by anatomy[60], so are the electrographic and behavioral responses to epileptogenic insults (Fig. 4).

In most SE models, subcortical and temporal cortical damage is thought to drive seizure generating network(s).[61] In many of the TBI and stroke induced epilepsy models, however, the seizure onset zone is likely located in the cortico-thalamo-cortical network, although comprehensive studies remain to be done.[62-66] The hippocampus, amygdala, piriform and entorhinal cortices are low-threshold entry points into the limbic seizure circuit.[67,68] Conceptually, nodes of this network can be thought of as drivers (e.g., cortical regions, hippocampus, amygdala) and distributors (e.g., midline thalamus) that relay ictal activity from the driver focus to a broader network (Fig. 4A). The thalamocortical network has been best examined with absence seizures: corticothalamic drivers recruit intrathalamic distributors which in turn recruit thalamocortical drivers (Fig. 4B).

While the nonconvulsive focal seizures reported in acquired models typically have a higher frequency than that of genetic absence bilateral seizures, they might engage this same circuits.[64, 69, 70] In both of these circuits, primary components may be modulated by inputs that, while not central to ictogenesis, can influence patterns of excitation and/or gate seizure activity.

Perilesional focal seizures or pathological high frequency oscillations (HFOs) at the site of injury often predominate in the early post-injury phase and precede the development of limbic or thalamocortical spreading seizures.[20,71,72] While HFOs are behaviorally silent (i.e., purely electrographic), non-spreading perilesional focal seizures induce behavioral arrest when occurring in the frontal neocortex[63], but appear behaviorally silent when occurring in the parietal or occipital neocortex[72,73]. The emergence of seizure activity beyond the injury site may be thought of as secondary epileptogenesis.

Seizure activity evoked by direct activation of cortical drivers gains rapid access to the limbic seizure network by virtue of cortico-cortical projections, cortico-thalamic projections and projections from cortex to subcortical drivers.[74-77] Similarly, subcortical foci recruit cortical seizure involvement through direct projections and through distributors.[78,79] Cortical

foci may likewise recruit the thalamocortical circuit.[80] In many models for example, focal suppression of activity in the thalamus is potently anticonvulsant[64, 79, 81, 82] and the same is true in patients with drug resistant types of focal epilepsy[83].

Direct injury to cortical drivers after TBI - frontal, parietal, or occipital-directed FPI- all trigger pathology in hippocampus, and are associated with both limbic seizures and thalamocortical-like seizures that present -just like in humans- different clinical correlates depending on the ictal sites.[70, 71,73] Suppression of ventrobasal neuron activity after cortical infarct inhibits seizures both in the cortex and thalamus[64], suggesting that just as in some evoked models (e.g., cortical penicillin), activity in the thalamus is necessary to maintain cortical seizures.[80]

Direct injury to sub-cortical drivers (e.g. hippocampus after TBI) is unsurprisingly associated with primary seizure onset zones in the hippocampus. However, seizure onset has also been reported in other nodes of the network, including cortical drivers such as piriform cortex.[84] This pattern is similar in rats and humans, where even within a single subject, seizures may initiate at different foci.[85-87] Direct injury to cortical drivers after TBI is particularly informative - frontal, parietal, or occipital-directed FPI *all* trigger pathology in hippocampus, and are *all* associated with both limbic seizures and thalamocortical-like seizures.[71,73] Primary injury to the hippocampus after focal herpes encephalitis[88-90] or Theiler's murine viral encephalitis [91-93] is likewise associated with hippocampal seizures[94]. Secondary injury to subcortical drivers is also seen after TBI, controlled cortical impact (CCI), and photothrombotic CVA lesions. These changes in hippocampus along with primary cortical injury may underlie the decreased resting state BOLD[95] and the decreased cortical and hippocampal glucose utilization reported after FPI[96].

In addition to the temporal lobe seizure phenotype seen in some models, several models of acquired epilepsy display non-convulsive focal seizures. Brief focal seizures, with accompanying behavioral arrest have been reported after FPI, ballistic injury, and ischemic

injury in rats.[64, 97-100] These are associated with injury to cortical drivers[62, 63,70] and alterations in gene expression and physiology in intrathalamic distributors and drivers.[101] The relevance of these discharges to acquired epilepsies in humans is debated.[72,102,103]

Cases in which seizures originate from sites distal to the injury are perhaps the most interesting and suggest that insults can exert epileptogenic effects by engaging a constrained set of networks. While a great deal is known regarding these networks in evoked seizures and classic models, little network mapping has been conducted following TBI, hypoxia/ischemia, or infection. Even fewer studies have directly manipulated the primary seizure circuitry in these models[64], and *no* studies have manipulated modulators (e.g., basal ganglia, cerebellum) after these insults, although they gate a wide variety of seizures in other models.[104-107] These areas of future exploration may prove essential to determining the degree of network overlap among these models and other models of acute or acquired epilepsy, and may likewise guide network level interventions for acquired epilepsies.

Commonalities in circuit reorganization after acquired brain injuries

Acquired (but also inherited) brain injuries result in complex network reorganizations. To paraphrase Eccles, Ito and Szentágothai's classic book, one can see "Epilepsy as a neuronal plasticity machine". In fact, it is difficult to pinpoint a network parameter that is *not* changed in epilepsy. Moreover, other than those with epileptic encephalopathies, seizures are usually rare events even in people with severe epilepsy (a person with daily seizures will still spend 99.9% of the time not having clinical seizures), pointing to the existence of compensatory mechanisms, which, most of the time, prevent clinical seizures from occurring. Electrophysiological changes in epileptogenesis and ictogenesis are therefore difficult to interpret, as they could constitute compensatory mechanisms, reflecting a different organization of neuronal circuits to carry the same tasks.[108]

The most obvious reorganizations commonly found in patients and animal SE models

of epilepsy are cell loss, particularly of GABAergic interneurons[109-111] reactive

synaptogenesis and axonal sprouting, in particular from glutamatergic neurons[112-115], and the

molecular reorganization of glutamate and GABA receptor subunits.[116-118] Many of these

features are also found in TBI models, such as the sprouting of excitatory axons, the increase

in excitatory drive and decrease in inhibitory drive.[119,120] However, some changes, for

example, GABA subunit changes during epileptogenesis, may be insult-specific.[121] Whether

these modifications are similar in animal models and patients is difficult to address. In

humans, the lack of proper control tissue (peritumoral non-epileptogenic tissue is usually used

as control) prevents an appropriate comparison. Despite this caveat, commonalities are clearly

apparent. Do these changes cause similar network consequences?

In rodent models and humans, a loss of interneurons results in decreased GABAergic

synaptic (phasic) inputs.[122,123] However, phasic inhibition is decreased in the dendrites but

increased in the soma in pyramidal cells[123], despite a reduction in GABA quantal release.[124]

GABA also exerts tonic inhibition of neurons via activation of extrasynaptic receptors. In

animal post-SE epilepsy, TBI and stroke models and in human epilepsy, there is a

preservation (or indeed an increase) in tonic inhibition, despite the decrease in phasic

inhibition.[125-128] The computational consequences of the shift of GABAergic inhibition from

dendritic to somatic results in inhibitory drive being more capable of synchronising principal

cells, and the shift of inhibition from phasic to tonic results in an increase in the gain of

neurons, whilst preserving their baseline.[123,129] The predicted net result is an increase in

synchronisation and excitability during periods of increased network activity. Large scale

realistic computer models now exist to test this prediction directly.[130]

The inhibitory action of GABA depends upon the reversal potential of Cl^- currents

(E_{Cl}). A minority of neurons in human slices show altered E_{Cl} making GABA

depolarizing[131,132], a result reproduced in rodent models but only before animals developed

spontaneous seizures[133]. Interictal activity appears to depend upon depolarizing GABA *in*

vitro.[131,132] In experimental models *in vivo*, interictal activity originates from interneuron firing.[134] Interneurons may play opposing roles during the progression and spread of seizure activity, and these roles may be determined both by the synchronising effects of somatic inhibition and the propensity for inhibition to become depolarising during excessive activity.[135,136] Glutamatergic drive is also increased in epileptogenic tissue from humans[137] and rodents[115], with an increased contribution of NMDA receptors, which contribute to the bursting response.[138-140]

Finally, acquired channelopathies, which could change the excitability and integrative properties of cells, have been described in experimental models.[141,142] Interestingly, genetic overexpression of Kv1.1 K^+ channels can be disease modifying in experimental models.[143] These issues remain to be investigated in human tissue.

Commonalities in electrophysiological alterations extend to seizures themselves. Seizure dynamics appear to follow general rules across species (from flies to humans) and brain regions[144], but whether electrophysiological commonalities can "explain" epilepsy is not known, as, for most, no causal link has been established. There are many different biophysical paths to seizures in any given neuronal circuit[144], and it is likely that, in most cases, the electrophysiological changes that occur just reflect lowered threshold for seizure genesis and/or facilitated seizure propagation.[145]

Common hippocampal and parahippocampal circuits and structural lesions

Many animal models of epileptogenesis involve structural etiologies, *e.g.*, TBI, stroke, and cortical dysplasia.[146] A variety of seemingly unrelated insults, including head trauma, prolonged febrile seizures, vascular abnormalities and encephalitis, can cause similar hippocampal pathology[43,147-149] and the same seizure disorder, i.e. acquired mesial TLE with hippocampal sclerosis.[150,151] The hippocampal and temporal cortical damage after experimental SE resembles that found in resected tissue of patients with refractory TLE of different

etiologies, including loss of dentate hilar and hippocampal pyramidal cells, layer III damage in the entorhinal cortex, gliosis, and mossy fiber sprouting.[152-154] Notably, dentate hilar neuron loss (endfolium sclerosis) was reported to be the single common pathology in epilepsy patients with any detectable hippocampal pathology.[147]

TBI models induced with CCI or lateral FPI result in loss of hilar cells, mossy fiber sprouting, gliosis and neurogenesis, whereas granule and pyramidal cell damage is less severe than in SE models.[155] Hippocampal inhibitory neurons appear particularly vulnerable to lateral FPI.[156] In correspondence with animal models, a subpopulation of patients with TBI shows progressive hippocampal volume loss and white matter atrophy in MRI.[157,158] Taken together, hippocampal atrophy is often seen in acquired epilepsy patients and experimental models. However no study has compared hippocampal pathology between experimental models or humans with or without post-SE, post-TBI or post-stroke epilepsy, which is critical to pinpoint epileptogenesis-specific pathologies.[8]

In some rodent models, prolonged dentate granule cell discharge selectively destroys vulnerable hippocampal neurons that control granule cell inhibition[109,159], thus weakening the normal gating function of the dentate gyrus.[161-165] Mild to moderate fluid percussion injury can also cause selective hilar neuron loss and granule cell hyperexcitability.[166] Why do dissimilar insults sometimes produce similar pathology and the same neurological condition?

A central role has been proposed for excitatory dentate hilar mossy cells in establishing granule cell inhibition via activation of inhibitory neurons[159,160], and for mossy cell loss in granule cell disinhibition[109,152,165]. Optogenetic activation or silencing of dentate granule cells promoted or suppressed seizures in a unilateral hippocampal kainate mouse model, respectively[106], in support of the dentate gate hypothesis. Similar studies capable of selectively exciting or silencing residual mossy cells are needed to refute or confirm the "dormant basket cell" hypothesis.[152,167] Regardless of the network mechanism underlying injury-related granule cell hyperexcitability, the selective loss of entorhinal cortex Layer III neurons[168] and dentate

hilar neurons[159] may be a common pathology produced by different insults.

An alternate perspective holds that epileptogenesis requires prolonged molecular and/or structural changes triggered by acute brain injury, because epilepsy develops only after a seizure-free period that can last for decades.[169] In this scenario, during the period from insult to clinical epilepsy the epileptogenic mechanism gradually elaborates.[66,170,172] Hippocampal transcriptomics and miRNA changes in SE and TBI models and in human TLE have been studied in an effort to identify critical pathways (Fig. 5 and Supplementary Tables 1 and 2). Very few similarities have been identified in hippocampal transcriptomics and miRNA networks among the various structural models, even when data from the same etiology (e.g., SE models) are compared, or between the experimental data and the sparse data available from human epileptic hippocampus. However, comparison of these findings has been difficult because of variability in cell types, tissue sampling, timing of tissue sampling during the course of epileptogenic process, analysis platforms, brain regions, methods for inducing injury, age and species of animals, and unknown epilepsy phenotype of animals sampled at early time points. Dingledine et al. overcame some of the methodological difficulties.[172] They performed a well-powered transcriptomics analysis of laser-dissected dentate granule cells in three different SE models, each model generated in two laboratories, used the same analysis platform, and found only 4.5% concordance of differentially expressed genes among the models.

The same hippocampal network that can serve as a primary seizure source when the hippocampal formation is injured may play a secondary role when seizure activity from extratemporal foci spreads to, and recruits, the hippocampal formation.[173] Several brain regions innervate and influence the hippocampal formation, including non-temporal neocortex, thalamus, amygdala, and septum[174], and it is likely that both the damaged and undamaged hippocampus can generate the same clinical signs regardless of where seizures start. Seizures that originate from temporal neocortical- and even extra-temporal neocortical foci may

propagate to the hippocampal formation in both humans[175,176] and rodents[71].

In sum, qualitative similarities in hippocampal pathology are evident among the SE, TBI, and stroke models as well as between experimental and human TLE triggered by these etiologies. These deserve further analysis in the context of search for biomarkers (Box 2, Supplementary Table 3 and Text 2) and treatment targets of epileptogenesis.

Dysfunctional astrocytes and microglia: commonalities in epileptogenic processes

A common pathological hallmark in the sequence of events converting a normal brain into an epileptic brain after an acquired insult is microglial activation and subsequent astrogliosis, which results from inflammatory processes.[177-180] Astrocytes regulate and amplify immune-mediated mechanisms implicated in epileptogenesis.[177,181-186] Evidence from experimental epileptogenesis studies and from studies of established human epilepsy shows that astrocytes undergo functional changes including dysregulation of astroglial K_{ir} and gap junction channels, which together alter glio-neuronal communication and, by impairing uptake and redistribution of extracellular K^+ accumulated during neuronal activity, can contribute to or cause seizures.[178,185-189] Downregulation of astrocytic K_{ir} channels appears to be a common response to brain injury and has been observed after TBI[190], CVA[191], BBB damage[192], entorhinal cortex lesions[193], and freeze-lesion induced cortical dysplasia[194,195], and in association with experimentally induced posttraumatic epilepsy.[70] Similarly, patch clamp analysis in neurosurgical specimens from TLE patients reveals reduction in astroglial K^+ currents in sclerotic vs. non-sclerotic hippocampi.[196] Reduced glial K^+ conductances and loss of astrocyte gap junction coupling compromise the clearance of extracellular K^+ and impair K^+ homeostasis. Loss of coupling is mediated by pro-inflammatory cytokines and precedes alterations in neurons and K^+ clearance, suggesting a causal role of dysfunctional K^+ buffering by astrocytes in the etiology of different epilepsies.[185,186] Reduced astrocyte coupling was also reported after acute CVA *in situ*.[197] Whether glial coupling is affected after

TBI is unknown. One study observed enhanced phosphorylation of Cx43 protein in astrocytes after experimental TBI[198] but functional coupling was not assessed.

Albumin extravasation into the brain following focal injury causes inflammation and activation of a TGF-β pathway in astrocytes, which impairs K^+ homeostasis and leads to epilepsy. Importantly, treatment with losartan, a clinically used angiotensin II type I receptor inhibitor, prevented epilepsy in most mice.[199] In addition to its known action as an angiotensin II type I receptor inhibitor, losartan exhibits effects on inflammatory pathways and potassium channels[200] that might contribute to its antiepileptogenesis effect.

Astrocytes control the availability of the brain's endogenous anticonvulsant, adenosine, through expression of ADK.[201] As a molecular link between energy homeostasis and metabolic demand, ADK expression undergoes acute downregulation followed by chronic overexpression, triggered by ischemia or TBI, and chemically or electrically induced SE.[57, 202-207] Overexpression of ADK is linked to astrogliosis and has been reported in human TLE surgical specimens with hippocampal sclerosis and in tumor-associated epilepsy.[205,208-210] Increased ADK lowers tissue levels of adenosine, thereby increasing neuronal excitability and precipitating seizures.[203,206,211] In addition, increased ADK promotes DNA methylation as an epigenetic contributor to epileptogenesis.[55,57] Overexpression of ADK has been associated with progression and worsening of epilepsy.[205] Conversely, therapeutic adenosine augmentation[212,213] suppresses not only seizures but also prevents development and progression of epilepsy in systemic kainate and pilocarpine models[57,214].

In conclusion, dysfunction of glial K_{ir} channels and gap junctions, and subsequent disruption of K^+ and adenosine homeostasis are common responses of the brain to TBI, CVA, SE, BBB damage, cortical lesion and dysplasia, and may contribute to or initiate epileptogenesis. Aquaporin 4, expressed exclusively in astrocytes, is translocated from perivascular astrocytic end-feet to the parenchyma in cortical tissue resected during epilepsy surgery.[215] A role for aquaporin 4 in the disruption of water balance during epileptogenesis is

worth following up.[216-218]

Inflammatory processes after acquired brain injuries

Brain inflammation, or neuroinflammation[219], is common after TBI, CNS infections, CVA and SE in humans and animals. Common to all these different acute injuries is the generation of an immune/inflammatory response in CNS-specific cells and cellular components of the BBB. A key common brain response is the rapid activation of microglia[220], which promptly release an array of frontline inflammatory molecules including damage-associated molecular patterns (e.g. HMGB1, ATP, S100β), cytokines (IL-1β, TNF-α, IL-6), various chemokines [221] and related effector pathways including COX-2/PGE2 and complement factors.[36] This shared tissue injury response might be triggered to activate homeostatic programs. However, if the ensuing inflammatory cascade persists, it leads to CNS dysfunction and neuropathology.[220] Non-immune cells like astrocytes and neurons, together with the BBB, play an important role in the immune responses to injuries.[36,179,222] As various inflammatory molecules contribute to the mechanisms of acute and chronic seizures by altering synaptic transmission and neuronal excitability[223] (Fig. 6), it is conceivable that neuroinflammation contributes to the development of various forms of acquired epilepsies. [36,175,224-226] Inflammatory mediators commonly present in human brain and related animal models after acute epileptogenic injuries are shown in Supplementary Table 4. Type and severity of injury determine the relative contribution of innate immunity and circulating leukocytes to increased inflammatory molecules, acute or secondary brain damage[227-229], and neuronal network excitability. While HMGB1 and IL-1β contribute to hyperexcitability and epilepsy progression after SE in rodents[230-232], IL-6 has a pivotal role in epileptogenesis induced by murine viral encephalitis[233]. While antiinflammatory drugs dampen epileptogenesis in animal models of SE or viral encephalitis[231-235], there is no information whether inflammation is involved in epileptogenesis after stroke or TBI[236,237], except for focal cooling preventing both cortical IL-

1 induction and epilepsy after TBI in rats[62]. There is, however, strong evidence that inflammation plays either a pathologic or beneficial role following TBI or stroke depending on the post-injury phase[238,239]. While TNF-α or CX3CR1 knock-out mice showed reduced functional deficits compared to wild-type mice acutely post-TBI, their functional recovery over time was impaired and cortical damage was increased[240-242]. Similarly, TNF-α was involved in both secondary brain damage and regenerative recovery after experimental stroke.[243-245] The pathologic involvement of inflammation is supported by evidence that excessive brain levels of IL-1β, IL-6, TNF-α (exceeding several times their serum changes) or HMGB1[232,246,247] contribute to experimental acute and chronic seizures and SE-induced epileptogenesis[36,179,226]. Importantly, HMGB1 antibodies have been shown to prevent epileptogenesis in the mouse intrahippocampal kainate model.[248] Studies of neuroinflammatory changes in postmortem human brain after CVA/stroke, TBI, infections and SE are limited, although they are essential to validate the potential pathophysiological role of injury-induced inflammation in epileptogenesis. Encephalitis-induced epilepsy caused by neurotropic viruses[226] is associated with activation of Toll-like receptors, which drives neuronal damage but may also promote tissue repair.[249] The precise relationship between viruses, inflammation and seizure development requires further studies in animals and humans.

We need to better understand the difference between pathologic vs. repair and recovery responses, and evaluate more thoroughly potential commonalities across brain injuries, in order to design drugs with broad therapeutic potential. The term "inflammation" covers many pathways some of which are beneficial after injury[238,239], such that targeted intervention at the right time could succeed when global suppression has failed.

Signaling pathway commonalities

Four pathways activated after several types of cerebral insults that are associated with

epileptogenesis or epilepsy progression are Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT), mammalian Target of Rapamycin Complex (mTORC), BDNF/tyrosine receptor kinase B/phospholipase Cγ1 (BDNF/trkB/PLCγ1), and IL-1R1/TLR4 pathways (Fig. 7). These signaling pathways mediate cellular physiological mechanisms that are critical for memory formation, long-term depression (LTD) and long-term potentiation (LTP).

The JAK/STAT pathway (Fig. 7A) is activated after many types of epileptogenic injuries in experimental models, including TBI, SE and stroke.[250-255] It regulates expression of genes critical for multiple essential functions including cell proliferation, differentiation, neurogenesis, learning and memory. JAK/STAT mediates decreases in α1 subunit-containing GABA$_A$ receptors in hippocampus that occur after experimental SE and TBI [250,252] and may contribute to the increased hyperexcitability observed in the hippocampus of injured animals and to subsequent development of epilepsy. Inhibition of STAT3 phosphorylation after SE-induced brain injury reduces the severity of subsequent epilepsy in the rat pilocarpine model[256], suggesting that JAK-STAT activation after SE may contribute to epileptogenesis. In the CCI model of PTE in mice, the JAK/STAT pathway is differentially activated depending on the severity of brain injury, and correlates with the presence and severity of subsequent cognitive co-morbidities.[257] Interestingly, transient inhibition of the JAK/STAT pathway immediately after CCI improved long-term vestibular motor recovery after injury but did not significantly affect memory performance.[257]

In rodent models, epileptogenic brain injuries including SE and TBI trigger an immediate and long-lasting increase in the phosphorylation of the S6 ribosomal protein in the hippocampus, which is a downstream marker of an excessively activated mTORC1 pathway (Fig. 7A).[258-260] Similarly, structural or dysplastic lesions associated with infantile spasms in rodents or humans also demonstrate overactivation of the mTORC1 pathway in the epileptogenic area.[261] Treatment with mTORC1 inhibitors is anti-seizure[262-264] and in some

models of acquired epilepsy, particularly tuberous sclerosis, antiepileptogenic.[259,265-267] Excessive activation of the mTORC1 pathway in acquired models of epilepsy is also associated with cognitive and behavioral deficits, which may be reversed following treatment with the mTORC inhibitor rapamycin.[258,266] Taken together, these findings suggest that JAK-STAT and mTORC1 signaling pathway activation following a variety of brain injuries may contribute to development of acquired epilepsy and co-morbid hippocampal-dependent spatial learning and memory deficits.

The interaction of BDNF with receptor trkB is involved in fundamental cellular processes including neuronal proliferation, differentiation and survival as well as neurotransmitter release and synaptic plasticity. Abnormal BDNF-mediated activation of TrkB has been reported in various neurological and psychiatric disorders.[268] Altered TrkB signaling (Fig. 7B) underlies epileptogenesis in both the kindling model[35] and after SE, as demonstrated by the ability of TrkB inhibitors targeting the PLCγ1 signaling pathway that were administered during the latent period to prevent the development of acquired epilepsy in some animals.[100,269] The IL-1R1/TLR4 pathway (Fig. 7A), activated by IL-1β and HMGB1, contributes not only to acute and chronic seizure recurrence[270] and kindling[271] but also to disease progression post-SE in rodents.[231, 232,247]

The pharmacology of animal models of acquired epilepsy

The rat amygdala kindling model (AKM) of TLE has been extensively studied [6,272,273], although kindled rats do not exhibit SRS, and therefore lack a defining feature of human TLE[274]. Nonetheless, drugs effective in human TLE are effective against seizures in fully kindled rats.[275] The efficacy of various anti-seizure drugs (ASDs) in suppressing seizures in fully kindled rats is remarkably similar to their efficacy in reducing the frequency of SRS in post-SE TLE models(Table 2).[276] About 20% of kindled rats are resistant to high doses of ASDs such as phenytoin. Similarly, ASD-resistant rats can be selected from post-SE models

of TLE.[275] The pharmacology of SRS developing in models of TBI, stroke or viral encephalitis is largely unknown, with some exceptions.[277-279] In models of TBI and encephalitis, valproate reduced seizure burden while carbamazepine was ineffective, indicating pharmacologic commonalities.[277,279]

Drug treatment during amygdala kindling acquisition has been used to detect disease-modifying efficacy by administering drug before each amygdala stimulation. Retardation of kindling development may indicate a true antiepileptogenic action or simply suppression of the effects of the stimulation. For most ASDs the latter is true, whereas for some drugs, including valproate, levetiracetam, and brivaracetam, effects on kindling acquisition (e.g., reduced seizure duration) persist long after drug wash-out, suggesting enduring disease modification.[6,280-282] The disease-modifying effects of such drugs have been confirmed in post-SE models of TLE[6], suggesting that there are shared mechanisms of epileptogenesis in the kindling and SE models.

A variety of experimental and clinically approved drugs has been evaluated for antiepileptogenic effects in post-SE models of TLE.[6, 275, 282, 283] Among clinically used ASDs, disease-modifying effects (e.g., neuroprotection, prevention of behavioral comorbidities, reduced frequency, duration or severity of SRS) were only detected after treatment with valproate, levetiracetam, brivaracetam or topiramate, although none of these drugs prevented epilepsy.[6, 282] While SE models involve significant neuronal loss, neuroprotection alone may be disease-modifying but not antiepileptogenic.[6] Some studies found that antiinflammatory drugs such as celecoxib or immunosupressive drugs such as rapamycin exert antiepileptogenic activity in post-SE models, but these effects appear to be very sensitive to the antiinflammatory drugs used and the timing of drug administration.[6, 261, 282, 284] Interestingly, bumetanide, which inhibits the Na-K-2Cl importer NKCC1[285] and thus increases the hyperpolarizing action of GABA, was not antiepileptogenic in the lithium-pilocarpine SE model in adult rats[286] although it was reported to have antiepileptogenic

actions in a model of complex febrile seizures in rats[287] and in a mouse model of genetic epilepsy[288]. By contrast, in SE models a trkB signaling inhibitor[100,269], the multitargeted drug isoflurane, which exhibits antiinflammatory, antioxidative, antiapoptotic, and neuroprotective effects[289], and HMGB1 antibodies[248] can be antiepileptogenic, providing crucial proof-of-principle that antiepileptogenesis is possible. In addition, inhibition of the IL-1R1/TLR4 pathway, and the associated ROS production, has been shown to mediate disease modifying effects in different post-SE models.[231,232,247]

Only a few studies used TBI models to evaluate possible PTE prevention by drug treatment during the latent period.[9] When PTE was induced by rostral parasagittal FPI in rats, 5 weeks of mild focal cooling of the perilesional neocortex almost completely prevented the development of epilepsy[62], whereas he investigational drug carisbamate exerted no antiepileptogenic efficacy[278], indicating that the model is capable of detecting antiepileptogenic activity. As noted, losartan prevented epileptogenesis in a PTE model, albeit in high doses.[199]

Other studies report antiepileptogenic efficacy of statins in different models[290], and of minocycline in an encephalomyelitis virus infection model[291]. The preclinical effects of statins were translated to a clinical study. Guo et al.[259] reported that acute statin use reduces the risk of poststroke early-onset seizures, and may also prevent the progression of initial poststroke seizures to chronic epilepsy, findings that, if reproducible, would be the first proof-of-concept of antiepileptogenesis in humans.[292] Antiepileptogenic effects of statins are also suggested by two other clinical studies.[293, 294] In addition to effects on serum cholesterol levels, statins exhibit immunomodulatory, antiinflammatory, and anti-excitotoxic properties[290], which could explain the antiepileptogenic effects that have been observed both experimentally and clinically.

Consequences for translation and development of antiepileptogenic therapies

There are three key questions. First, which of the commonalities described in neuropathology, EEG parameters, and molecular and inflammatory pathways are causes rather than consequences of acquired epilepsy? Second, are these commonalities able to clearly distinguish the patients who develop epilepsy from those who have a similar brain insult, but do not develop epilepsy? Third, to what extent do potential causal or susceptibility genes impact the propensity for developing acquired epilepsy? Biomarkers of impending epilepsy are needed to design feasible clinical trials to address these questions. Nonetheless, the richness of commonalities described in this review supports the potential benefit of combinatorial drug treatment, as already demonstrated in preclinical antiepileptogenesis studies.[295] Furthermore, these commonalities also appear to emphasize the promise of systems and network approaches towards identification of master regulators controlling epileptogenesis that may provide drug targets for future antiepileptogenic therapies.[296-298]

The first step in clinical development of antiepileptogenic therapies would be assessment of their safety and tolerability in healthy volunteers, a standard approach for Phase I studies. It will be advantageous to demonstrate target engagement in the brain before moving into later stages, in order to reduce the risk of development failure.[299,300] A newly developed synaptic density marker appears promising to measure neurodegeneration and neuronal network changes during epileptogenesis.[301] Other indirect target engagement measures could include quantitative EEG or MEG techniques[302] or assessment of specific molecular biomarkers in plasma that are clearly linked with drug activity in the brain. Development of such clinical biomarkers (Box 2) should be performed in parallel with the preclinical drug development of antiepileptogenic drug candidates.

Antiepileptogenesis studies should be performed in homogenous patient cohorts sharing similar clinical features such as, for example, severity of injury, accumulation of intracranial blood and incidence of early seizures, because these appear to be common and most likely predictors of focal epilepsy development (Table 3). Other important stratification

27

factors should include the commonality in early EEG parameters (e.g. HFOs) and similarities in the involvement of the epileptogenic limbic structures (e.g. MRI). Furthermore, whenever possible, stratification based on molecular biomarkers should be applied (Box 2). Subjects sharing involvement of similar mechanisms such as dysregulation of the JAK-STAT, mTOR, or BDNF/trkB pathways and neuroinflammation are preferred for antiepileptogenesis trials. The importance and availability of relevant disease-specific and drug response biomarkers is critical for success in clinical development. A recent analysis of outcome of more than 1000 clinical development programs, performed between 2006 and 2015, showed that clinical development success leading to regulatory approval is three times higher when biomarkers permit patient stratification.[303] Finally, specific causal or susceptibility genes, if properly validated in enabling studies, should be the ultimate stratification factors since their value was already demonstrated in trials targeting disease modification in other neurodegenerative diseases.[304]

Depending on the clinical development strategy, proof-of-concept studies should accumulate evidence that the onset of epilepsy is delayed after brain injury (ideally prevention of epilepsy) or that core clinical features of an ongoing epilepsy are substantially altered (disease modification). The standard ASD add-on trial design and seizure outcome may be used, the difference being that the follow-up period may need to be much longer in prevention trials to demonstrate a therapeutic effect after withdrawal of the antiepileptogenic agent.[3,283,305]

Clinical development of drug combinations is associated with a number of significant challenges, therefore the Food and Drug Administration (FDA) issued practical guidelines to facilitate this process.[306,307] It is certainly more straightforward to develop a combination treatment that consists of already approved medications, than for two or more investigational drugs used in combination. However, regardless of the approval status of the drug constituents, the New Drug Application (NDA) submitted to the FDA should contain

appropriate data to establish the overall safety and effectiveness for the new dosing regimen or indication as proposed in the combination product. Even in the case of already approved drugs such data may not be easily obtainable or compatible with the proposed drug combination, new indication or treatment regimen.

The biggest challenge in development of combinatorial drug treatments is the need to demonstrate that each constituent contributes to the efficacy. This typically involves the necessity to include single drug arms in the trial, which considerably increases the complexity of the design, overall study duration and costs. It is even more challenging if the optimal doses of each drug are not yet defined, which may require additional dose-finding studies for each drug alone or in combination. Finally, even before embarking on clinical trials additional preclinical data may be needed, e.g., safety/toxicology, pharmacokinetics (drug-drug interactions), target/biomarker interactions, etc.

Lastly, there may be legal, intellectual property and freedom-to-operate limitations, particularly for approved drugs used in combination. This may further complicate the development and commercialization of the new combinatorial product.

Conclusions and future directions

The data reviewed indicate that there are a number of commonalities among the three main acute CNS injuries that lead to epilepsy in humans – TBI, CVA and infection. At the clinical level, the presence of intracranial blood, breakdown of the BBB, severe injury, early clinical seizures and EEG seizures, and periodic discharges on early EEG increase the risk for epilepsy in all three. Common anatomical modifications include astrocytosis, white matter abnormalities impacting seizure network and propagation, and neuronal heterotopia. Common elements in animal models include involvement of anatomically constrained networks that relay and distribute seizures remotely from the initial locus of injury, which can explain the common phenomenon of secondary epileptogenic involvement of the temporal lobe,

activation of astrocytes with impairment of extracellular K^+ buffering and of adenosine homeostasis, selective death of inhibitory interneurons and disinhibition/overinhibition of primary excitatory cells, and activation of the JAK/STAT and mTORC1 pathways. On the other hand transcriptomics have as yet shown few shared gene expression changes across (or within) models, and the role of neuroinflammation and BDNF/Trk signaling in epileptogensis has been demonstrated in SE and kindling models but has not yet been evaluated in animal PTE and PSE. Whether the causes of epilepsy share common mechanisms and features with the causes of the most common comorbidities (anxiety, depression) is an important question to pursue.

Review of the data suggests several conclusions: 1.The three common causes of epilepsy after acute injury, TBI, CVA and infection, share enough risk factors, electrophysiology, and pathology features to possibly consider them, in epileptogenesis, as one broad category (with individual variations). This has major implication for preventive therapeutic development. To validate or disprove this hypothesis, we should target comparison of epileptogenic processes as well as potential biomarker discovery and therapeutic intervention in both clinical and animal studies comparatively across the three types of injury - particularly for TBI and CVA where clinical intervention is logistically most feasible. If the hypothesis proves correct, this would allow a broader approach to development of biomarkers of epileptogenesis and of preventive treatments.

2. It is still unclear whether data obtained from the logistically easier models of SE and kindling are applicable to the human conditions of TBI, CVA and infection because relevant data do not exist. This calls for a concerted effort to systematically evaluate the SE and kindling model findings in the PTE, PSE and infection models. Answering this question will determine whether biomarker and therapeutic intervention studies, which can more easily be done in SE and kindling models, have relevance for human preventive treatment discovery. If they do, this would facilitate translation from animal to clinical studies.

3. Finally, this review identifies a plethora of potential biomarkers, as well as preclinical evidence for several preventive therapeutic strategies that can be followed in human studies. Clinical preventive studies may be significantly strengthened if the preclinical studies were systematically evaluated across the different animal models. Perhaps epileptogenic features, biomarkers and positive treatments common to all the animal models may have the greatest chance of clinical success, and lead, at long last, to a breakthrough in the preventive and/or disease modifying treatment for epilepsy.

Disclosure

P.K. has served on advisory boards of Lundbeck and UCB Pharma, has acted as a consultant to Eisai, Lundbeck and UCB Pharma, is on Speaker's Bureau of Eisai, Sunovion and UCB Pharma, and has received research grants from Eisai and Lundbeck. P.L.P. receives royalties from Demos Medical Publishers for the books, Inherited Metabolic Epilepsies and Neuro-Logic: A Primer on Localization. D.B. has acted as consultant to UCB Pharma and Roche. M.J.B. serves on the scientific advisory boards of Eisai, UCB Pharma, GlaxoSmithKline, Lundbeck, Bial, GW Pharmaceuticals and Takeda, is on the speakers' bureau for Eisai, UCB Pharma, GlaxoSmithKline, Lundbeck, Newbridge, Sanofi Aventis and Abbott and has accepted travel grants for scientific meetings from Eisai, UCB Pharma and Lundbeck. L.J.H. has received research support to Yale University for investigator-initiated studies from Eisai and Upsher-Smith; consultation fees for advising from Ceribell, Monteris, Neuropace, Sun Neuroscience, and Engage Therapeutics; royalties for authoring chapters for UpToDate-Neurology, chapters for Medlink—Neurology, and from Wiley for co-authoring the book "Atlas of EEG in Critical Care", by Hirsch and Brenner; and honoraria for speaking from Neuropace. A.P. has received research funding from Neurotrauma Sciences LLC. A.V. has

References

1   Banerjee PN, Filippi D, Allen Hauser W. The descriptive epidemiology of epilepsy-a review. Epilepsy Res. 2009; 85: 31–45.

2   Hauser WA, Annegers JF, Kurland LT. Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1993. Epilepsia; 34: 453–68.

3   Löscher W, Klitgaard H, Twyman RE, et al. New avenues for anti-epileptic drug discovery and development. Nat. Rev. Drug Discov. 2013; 12: 757–76.

4   Pitkänen A, Lukasiuk K, Dudek FE, et al. Epileptogenesis. Cold Spring Harb. Perspect. Med. 2015; 5: pii: a022822.

5   Temkin NR. Preventing and treating posttraumatic seizures: the human experience. Epilepsia 2009; 50 Suppl 2: 10–3.

6   Löscher W, Brandt C. Prevention or modification of epileptogenesis after brain insults: experimental approaches and translational research. Pharmacol. Rev. 2010; 62: 668–700.

7   Klein P, Tyrlikova I. Prevention of Epilepsy: Should We Be Avoiding Clinical Trials? Epilepsy Behav 2017; 72 : 188-94.

8   Pitkänen A, Kharatishvili I, Karhunen H, et al. Epileptogenesis in experimental models. Epilepsia 2007; 48 Suppl 2: 13–20.

9   Pitkänen A, Bolkvadze T, Immonen R. Anti-epileptogenesis in rodent post-traumatic epilepsy models. Neurosci. Lett. 2011; 497: 163–171.

10   Curia G, Eastman CL, Miller JW, et al. Modeling Post-Traumatic Epilepsy for Therapy Development. 2016. Translational Research in Traumatic Brain Injury. Boca Raton (FL): CRC Press/Taylor and Francis Group; 2016. Chapter 10.

11   Eastman CL, Fender JS, Temkin NR, et al. Optimized methods for epilepsy therapy development using an etiologically realistic model of focal epilepsy in the rat. Exp. Neurol. 2015; 264: 150–62.

12   Sinha S, Hirsch L, Ebersole J, et al. EEG monitoring in the ICU. In: Current Practice of Clinical EEG. Wolters Kluwer Health; 2014. p. 543–598.Siniscalchi A, Mintzer S. Statins for poststroke seizures: the first antiepileptogenic agent? Neurology 2015; 85: 661–2.

13   Herman ST, Abend NS, Bleck TP, et al. Consensus statement on continuous EEG in critically ill adults and children, part I: indications. J. Clin. Neurophysiol. 2015; 32: 87–95.

14   Dreier JP, Fabricius M, Ayata C, et al. Recording, analysis, and interpretation of spreading depolarizations in neurointensive care: Review and recommendations of the COSBID research group. J. Cereb. Blood Flow Metab. 2016, Jan 1:271678X16654496.

15   Löscher W, Hirsch LJ, Schmidt D. The enigma of the latent period in the development of symptomatic acquired epilepsy - Traditional view versus new concepts. Epilepsy Behav. 2015; 52: 78–92.

16   Wagenman KL, Blake TP, Sanchez SM, et al. Electrographic status epilepticus and long-term outcome in critically ill children. Neurology 2014; 82: 396–404.

17   Punia V, Garcia CG, Hantus S. Incidence of recurrent seizures following hospital discharge in patients with LPDs (PLEDs). Epilepsy Behav. 2015 Aug;49:250-4

18   Vespa P, Tubi M, Claassen J, et al. Metabolic crisis occurs with seizures and periodic discharges after brain trauma. Ann. Neurol. 2016; 79: 579–90.

19   Waziri A, Claassen J, Stuart RM, et al. Intracortical electroencephalography in acute brain injury. Ann. Neurol. 2009; 66: 366–77.

20   Bragin A, Li L, Almajano J, et al. Pathologic electrographic changes after experimental traumatic brain injury. Epilepsia 2016; 57: 735–45.

21   El Achkar CM, Olson HE, Poduri A, et al. The genetics of the epilepsies. Curr. Neurol. Neurosci. Rep. 2015; 15: 39.

22   McTague A, Howell KB, Cross JH, et al. The genetic landscape of the epileptic encephalopathies of infancy and childhood. Lancet. Neurol. 2016; 15: 304–16.

23   Epi4K. Ultra-rare genetic variation in common epilepsies: a case-control sequencing study Lancet Neurol. 2017; 16: 135–143.

24   Caveness WF, Meirowsky AM, Rish BL, et al. The nature of posttraumatic epilepsy. J. Neurosurg. 1979; 50: 545–53.

25   Frankel WN. Genetics of complex neurological disease: challenges and opportunities for modeling epilepsy in mice and rats. Trends Genet. 2009; 25: 361–7.

26   Glasscock E, Qian J, Yoo Jong, et al. Masking epilepsy by combining two epilepsy genes. Nature Neuroscience 2007. 10; 1554-1558

27   Jennett B. Epilepsy after non-missile head injuries. 2nd edition. London: Heinemann Medical; 1975.

28   Salazar AM, Jabbari B, Vance SC, et al. Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam Head Injury Study. Neurology 1985; 35: 1406–14.

29   Schaumann BA, Annegers JF, Johnson SB, et al. Family history of seizures in posttraumatic and alcohol-associated seizure disorders. Epilepsia; 35: 48–52.

30   Ottman R, Lee JH, Risch N, et al. Clinical indicators of genetic susceptibility to epilepsy. Epilepsia 1996; 37: 353–61.

31   Christensen J, Pedersen MG, Pedersen CB, et al. Long-term risk of epilepsy after traumatic brain injury in children and young adults: a population-based cohort study. Lancet (London, England)

32   Corey LA, Pellock JM, DeLorenzo RJ. Status epilepticus in a population-based Virginia twin sample. Epilepsia 2004; 45: 159–65.

33   Eckhaus J, Lawrence KM, Helbig I, et al. Genetics of febrile seizure subtypes and syndromes: a twin study. Epilepsy Res. 2013; 105: 103–9.

34   Bennett ER, Reuter-Rice K, Laskowitz DT. Genetic Influences in Traumatic Brain Injury. In: Laskowitz D, Grant G, editors. Translational Research in Traumatic Brain Injury. Boca Raton (FL)

35   McNamara JO, Scharfman HE. Temporal Lobe Epilepsy and the BDNF Receptor, TrkB. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Jasper's Basic Mechanisms of the Epilepsies [Internet]. 4th edition. Bethesda (MD)

36   Vezzani A, Friedman A, Dingledine RJ. The role of inflammation in epileptogenesis. Neuropharmacology. 2013b; 69:16-24.

37   Darrah SD, Miller MA, Ren D, et al. Genetic variability in glutamic acid decarboxylase genes: associations with post-traumatic seizures after severe TBI. Epilepsy Res. 2013; 103: 180–94.

38   Ritter AC, Kammerer CM, Brooks MM, et al. Genetic variation in neuronal glutamate transport genes and associations with posttraumatic seizure. Epilepsia 2016; 57: 984–93.

39   Wagner AK, Miller MA, Scanlon J, et al. Adenosine A1 receptor gene variants associated with post-traumatic seizures after severe TBI. Epilepsy Res. 2010; 90: 259–272.

40   Zhang B, Chen M, Yang H, et al. Evidence for involvement of the CD40/CD40L system in post-stroke epilepsy. Neurosci. Lett. 2014; 567: 6–10.

41   Yang H, Song Z, Yang G-P, et al. The ALDH2 rs671 Polymorphism Affects Post-Stroke Epilepsy Susceptibility and Plasma 4-HNE Levels. PLoS One 2014; 9: e109634.

42   Blümcke I, Spreafico R, Haaker G, et al. Histopathological findings in brain tissue obtained from epilepsy surgery. New Engl. J. Med. 2017; 377: 1648-1656.

43   Blümcke I, Sarnat HB, Coras R. Surgical Neuropathology of Focal Epilepsies: Textbook and Atlas. Montrouge, France: John Libbey Eurotext; 2015.

44   Garbelli R, Milesi G, Medici V, et al. Blurring in patients with temporal lobe epilepsy: clinical, high-field imaging and ultrastructural study. Brain 2012; 135: 2337–49.

45   Blümcke I, Thom M, Aronica E, et al. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. Epilepsia 2011; 52: 158–74.

46   Palmini A, Najm I, Avanzini G, et al. Terminology and classification of the cortical dysplasias. Neurology 2004; 62: S2-8.

47   Emery JA, Roper SN, Rojiani AM. White matter neuronal heterotopia in temporal lobe epilepsy: a morphometric and immunohistochemical study. J. Neuropathol. Exp. Neurol. 1997; 56: 1276–82.

48   Hildebrandt M, Pieper T, Winkler P, et al. Neuropathological spectrum of cortical dysplasia in children with severe focal epilepsies. Acta Neuropathol. 2005; 110: 1–11.

49   Rojiani AM, Emery JA, Anderson KJ, et al. Distribution of heterotopic neurons in normal hemispheric white matter: a morphometric analysis. J. Neuropathol. Exp. Neurol. 1996; 55: 178–83.

50   Hildebrandt M, Amann K, Schröder R, et al. White matter angiopathy is common in pediatric patients with intractable focal epilepsies. Epilepsia 2008; 49: 804–15.

51   Kobow K, Blümcke I. The methylation hypothesis: do epigenetic chromatin modifications play a role in epileptogenesis? Epilepsia 2011; 52 Suppl 4: 15–9.

52   Fischer A, Sananbenesi F, Wang X, et al. Recovery of learning and memory is associated with

chromatin remodelling. Nature 2007; 447: 178–82.

53 Peleg S, Sananbenesi F, Zovoilis A, et al. Altered histone acetylation is associated with age-dependent memory impairment in mice. Science 2010; 328: 753–6.

54 Dębski KJ, Pitkänen A, Puhakka N, et al. Etiology matters - Genomic DNA Methylation Patterns in Three Rat Models of Acquired Epilepsy. Sci. Rep. 2016; 6: 25668.

55 Williams-Karnesky RL, Sandau US, Lusardi TA, et al. Epigenetic changes induced by adenosine augmentation therapy prevent epileptogenesis. J. Clin. Invest. 2013; 123: 3552–63.

56 Kobow K, Jeske I, Hildebrandt M, et al. Increased reelin promoter methylation is associated with granule cell dispersion in human temporal lobe epilepsy. J. Neuropathol. Exp. Neurol. 2009; 68: 356–64.

57 Kobow K, Kaspi A, Harikrishnan KN, et al. Deep sequencing reveals increased DNA methylation in chronic rat epilepsy. Acta Neuropathol. 2013; 126: 741–56.

58 Da Silva FHL, Gorter JA, Wadman WJ. Epilepsy as a dynamic disease of neuronal networks. Handb. Clin. Neurol. 2012; 107: 35–62.

59 Kandratavicius L, Balista PA, Lopes-Aguiar C, et al. Animal models of epilepsy: use and limitations. Neuropsychiatr. Dis. Treat. 2014; 10: 1693–705.

60 Proix T, Bartolomei F, Guye M, et al. Individual brain structure and modelling predict seizure propagation. Brain. 2017; 140: 641-654.

61 Goldberg EM, Coulter DA. Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. Nat. Rev. Neurosci. 2013; 14: 337–49.

62 D'Ambrosio R, Eastman CL, Darvas F, et al. Mild passive focal cooling prevents epileptic seizures after head injury in rats. Ann. Neurol. 2013; 73: 199–209.

63 D'Ambrosio R, Hakimian S, Stewart T, et al. Functional definition of seizure provides new insight into post-traumatic epileptogenesis. Brain 2009; 132: 2805–21.

64 Paz JT, Davidson TJ, Frechette ES, et al. Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. Nat. Neurosci. 2013; 16: 64–70.

65 Paz JT, Huguenard JR. Microcircuits and their interactions in epilepsy: is the focus out of focus? Nat. Neurosci. 2015; 18: 351–9.

66 Bragin A, Wilson CL, Engel J. Chronic epileptogenesis requires development of a network of pathologically interconnected neuron clusters: a hypothesis. Epilepsia 2000; 41 Suppl 6: S144-52.

67 McIntyre DC, Kelly ME, Dufresne C. FAST and SLOW amygdala kindling rat strains: comparison of amygdala, hippocampal, piriform and perirhinal cortex kindling. Epilepsy Res. 1999; 35: 197–209.

68   Vismer MS, Forcelli PA, Skopin MD, et al. The piriform, perirhinal, and entorhinal cortex in seizure generation. Front. Neural Circuits 2015; 9: 27.

69   D'Ambrosio R, Fairbanks JP, Fender JS, et al. Post-traumatic epilepsy following fluid percussion injury in the rat. Brain 2004; 127: 304–14.

70   Stewart TH, Eastman CL, Groblewski PA, et al. Chronic dysfunction of astrocytic inwardly rectifying K+ channels specific to the neocortical epileptic focus after fluid percussion injury in the rat. J. Neurophysiol. 2010; 104: 3345–60.

71   D'Ambrosio R, Fender JS, Fairbanks JP, et al. Progression from frontal-parietal to mesial-temporal epilepsy after fluid percussion injury in the rat. Brain 2005; 128: 174–88.

72   Reid AY, Bragin A, Giza CC, et al. The progression of electrophysiologic abnormalities during epileptogenesis after experimental traumatic brain injury. Epilepsia 2016; 57: 1558–1567.

73   Curia G, Levitt M, Fender JS, et al. Impact of Injury Location and Severity on Posttraumatic Epilepsy in the Rat: Role of Frontal Neocortex. Cereb. Cortex 2011; 21: 1574–1592.

74   Poggio GF. The Propagation of Cortical-After-Discharge Through Subcortical Structures. Arch. Neurol. Psychiatry 1956; 75: 350.

75   Udvarhelyi GB. Dissemination of Acute Focal Seizures In the Monkey. Arch. Neurol. 1965; 12: 333.

76   Louis ED, Williamson PD, Darcey TM. Chronic focal epilepsy induced by microinjection of tetanus toxin into the cat motor cortex. Electroencephalogr. Clin. Neurophysiol. 1990; 75: 548–57.

77   Halonen T, Tortorella A, Zrebeet H, et al. Posterior piriform and perirhinal cortex relay seizures evoked from the area tempestas: role of excitatory and inhibitory amino acid receptors. Brain Res. 1994; 652: 145-148.

78   Walker AE. Dissemination of Acute Focal Seizures In the Monkey Arch. Neurol. 1965; 12: 357.

79   Bertram EH, Zhang D, Williamson JM. Multiple roles of midline dorsal thalamic nuclei in induction and spread of limbic seizures. Epilepsia 2008; 49: 256–68.

80   Avoli M, Gloor P. Interaction of cortex and thalamus in spike and wave discharges of feline generalized penicillin epilepsy. Exp. Neurol. 1982; 76: 196–217.

81   Cassidy RM, Gale K. Mediodorsal thalamus plays a critical role in the development of limbic motor seizures. J. Neurosci. 1998; 18: 9002–9.

82   Wicker E, Forcelli PA. Chemogenetic silencing of the midline and intralaminar thalamus blocks amygdala-kindled seizures. Exp. Neurol. 2016; 283: 404–12.

83   Fisher RS, Velasco AL. Electrical brain stimulation for epilepsy. Nat. Rev. Neurol. 2014; 10:

261-70.

84   Kendirli MT, Rose DT, Bertram EH. A model of posttraumatic epilepsy after penetrating brain injuries: effect of lesion size and metal fragments. Epilepsia 2014; 55: 1969–77.

85   Bertram EH. Functional anatomy of spontaneous seizures in a rat model of limbic epilepsy. Epilepsia 1997; 38: 95–105.

86   Wennberg R, Arruda F, Quesney LF, et al. Preeminence of Extrahippocampal Structures in the Generation of Mesial Temporal Seizures: Evidence from Human Depth Electrode Recordings. Epilepsia 2002; 43: 716–726.

87   Toyoda I, Bower MR, Leyva F, et al. Early activation of ventral hippocampus and subiculum during spontaneous seizures in a rat model of temporal lobe epilepsy. J Neurosci. 2013; 33:11100-15.

88   Schlitt M, Bucher AP, Stroop WG, et al. Neurovirulence in an experimental focal herpes encephalitis: relationship to observed seizures. Brain Res. 1988; 440: 293–8.

89   Stroop WG, Schaefer DC. Neurovirulence of two clonally related herpes simplex virus type 1 strains in a rabbit seizure model. J. Neuropathol. Exp. Neurol. 1989; 48: 171–83.

90   Chen S-F, Huang C-C, Wu H-M, et al. Seizure, neuron loss, and mossy fiber sprouting in herpes simplex virus type 1-infected organotypic hippocampal cultures. Epilepsia 2004; 45: 322–32.

91   Libbey JE, Kirkman NJ, Smith MCP, et al. Seizures following picornavirus infection. Epilepsia 2008; 49: 1066–1074.

92   Stewart K-AA, Wilcox KS, Fujinami RS, et al. Development of postinfection epilepsy after Theiler's virus infection of C57BL/6 mice. J. Neuropathol. Exp. Neurol. 2010; 69: 1210–9.

93   Bröer S, Käufer C, Haist V, et al. Brain inflammation, neurodegeneration and seizure development following picornavirus infection markedly differ among virus and mouse strains and substrains. Exp. Neurol. 2016; 279: 57–74.

94   Smeal RM, Stewart K-A, Iacob E, et al. The activity within the CA3 excitatory network during Theiler's virus encephalitis is distinct from that observed during chronic epilepsy. J. Neurovirol. 2012; 18: 30–44.

95   Mishra AM, Bai X, Sanganahalli BG, et al. Decreased resting functional connectivity after traumatic brain injury in the rat. PLoS One 2014; 9: e95280.

96   Shultz SR, Cardamone L, Liu YR, et al. Can structural or functional changes following traumatic brain injury in the rat predict epileptic outcome? Epilepsia 2013; 54: 1240–50.

97   Kelly KM, Kharlamov A, Hentosz TM, et al. Photothrombotic brain infarction results in seizure activity in aging Fischer 344 and Sprague Dawley rats. Epilepsy Res. 2001; 47: 189–203.

98   Hartings JA, Williams AJ, Tortella FC. Occurrence of nonconvulsive seizures, periodic

epileptiform discharges, and intermittent rhythmic delta activity in rat focal ischemia. Exp. Neurol. 2003; 179: 139–49.

99   D'Ambrosio R, Fairbanks JP, Fender JS, et al. Post-traumatic epilepsy following fluid percussion injury in the rat. Brain 2004; 127: 304–14.

100   Liu G, Gu B, He X-P, et al. Transient inhibition of TrkB kinase after status epilepticus prevents development of temporal lobe epilepsy. Neuron 2013; 79: 31–8.

101   Huusko N, Pitkänen A. Parvalbumin immunoreactivity and expression of GABAA receptor subunits in the thalamus after experimental TBI. Neuroscience 2014; 267: 30–45.

102   Rodgers KM, Dudek FE, Barth DS. Progressive, Seizure-Like, Spike-Wave Discharges Are Common in Both Injured and Uninjured Sprague-Dawley Rats: Implications for the Fluid Percussion Injury Model of Post-Traumatic Epilepsy. J. Neurosci. 2015; 35: 9194–204.

103   D'Ambrosio R, Eastman CL, Miller JW. Inadequate experimental methods and erroneous epilepsy diagnostic criteria result in confounding acquired focal epilepsy with genetic absence epilepsy. 2015

104   Iadarola MJ, Gale K. Substantia nigra: site of anticonvulsant activity mediated by gamma-aminobutyric acid. Science 1982; 218: 1237–40.

105   Bröer S, Backofen-Wehrhahn B, Bankstahl M, et al. Vigabatrin for focal drug delivery in epilepsy: bilateral microinfusion into the subthalamic nucleus is more effective than intranigral or systemic administration in a rat seizure model. Neurobiol. Dis. 2012; 46: 362–76.

106   Krook-Magnuson E, Szabo GG, Armstrong C, et al. Cerebellar Directed Optogenetic Intervention Inhibits Spontaneous Hippocampal Seizures in a Mouse Model of Temporal Lobe Epilepsy. eNeuro 2014; Dec;1(1). pii: e.2014.

107   Soper C, Wicker E, Kulick C V, et al. Optogenetic activation of superior colliculus neurons suppresses seizures originating in diverse brain networks. Neurobiol. Dis. 2016; 87: 102–15.

108   Marder E, Taylor AL. Multiple models to capture the variability in biological neurons and networks. Nat. Neurosci. 2011; 14: 133–8.

109   Sloviter RS. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. Science 1987; 235: 73–6.

110   de Lanerolle NC, Kim JH, Robbins RJ, et al. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res. 1989; 495: 387–95.

111   Houser CR, Esclapez M. Vulnerability and plasticity of the GABA system in the pilocarpine model of spontaneous recurrent seizures. Epilepsy Res. 1996; 26: 207–18.

112   Tauck DL, Nadler J V. Evidence of functional mossy fiber sprouting in hippocampal

formation of kainic acid-treated rats. J. Neurosci. 1985; 5: 1016–22.

113   Sutula T, Cascino G, Cavazos J, et al. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. Ann. Neurol. 1989; 26: 321–30.

114   Ben-Ari Y, Represa A. Brief seizure episodes induce long-term potentiation and mossy fibre sprouting in the hippocampus. Trends Neurosci. 1990; 13: 312–8.

115   Esclapez M, Hirsch JC, Ben-Ari Y, et al. Newly formed excitatory pathways provide a substrate for hyperexcitability in experimental temporal lobe epilepsy. J. Comp. Neurol. 1999; 408: 449–60.

116   Tsunashima K, Schwarzer C, Kirchmair E, et al. GABA(A) receptor subunits in the rat hippocampus III: altered messenger RNA expression in kainic acid-induced epilepsy. Neuroscience 1997; 80: 1019–32.

117   Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, et al. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. Nat. Med. 1998; 4: 1166–72.

118   Loup F, Wieser HG, Yonekawa Y, et al. Selective alterations in GABAA receptor subtypes in human temporal lobe epilepsy. J. Neurosci. 2000; 20: 5401–19.

119   Hunt RF, Scheff SW, Smith BN. Regionally localized recurrent excitation in the dentate gyrus of a cortical contusion model of posttraumatic epilepsy. J. Neurophysiol. 2010; 103: 1490–500.

120   Cantu D, Walker K, Andresen L, et al. Traumatic Brain Injury Increases Cortical Glutamate Network Activity by Compromising GABAergic Control. Cereb. Cortex 2015; 25: 2306–20.

121   Drexel M, Puhakka N, Kirchmair E, et al. Expression of GABA receptor subunits in the hippocampus and thalamus after experimental traumatic brain injury. Neuropharmacology 2015; 88: 122–133.

122   Williamson A, Patrylo PR, Spencer DD. Decrease in inhibition in dentate granule cells from patients with medial temporal lobe epilepsy. Ann. Neurol. 1999; 45: 92–9.

123   Cossart R, Dinocourt C, Hirsch JC, et al. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. Nat. Neurosci. 2001; 4: 52–62.

124   Hirsch JC, Agassandian C, Merchán-Pérez A, Ben-Ari Y, DeFelipe J, Esclapez M, et al. Deficit of quantal release of GABA in experimental models of temporal lobe epilepsy. Nat. Neurosci. 1999; 2: 499–500.

125   Scimemi A, Semyanov A, Sperk G, et al. Multiple and plastic receptors mediate tonic GABAA receptor currents in the hippocampus. J. Neurosci. 2005; 25: 10016–24.

126   Scimemi A, Andersson A, Heeroma JH, et al. Tonic GABA(A) receptor-mediated currents in

human brain. Eur. J. Neurosci. 2006; 24: 1157–60.

127   Clarkson AN, Huang BS, Macisaac SE, et al. Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature 2010; 468: 305–9.

128   Pavlov I, Huusko N, Drexel M, et al. Progressive loss of phasic, but not tonic, GABAA receptor-mediated inhibition in dentate granule cells in a model of post-traumatic epilepsy in rats. Neuroscience 2011; 194: 208–19.

129   Pavlov I, Savtchenko LP, Kullmann DM, et al. Outwardly rectifying tonically active GABAA receptors in pyramidal cells modulate neuronal offset, not gain. J. Neurosci. 2009; 29: 15341–50.

130   Alexander A, Maroso M, Soltesz I. Organization and control of epileptic circuits in temporal lobe epilepsy. Prog Brain Res. 2016, 226:127-54.

131   Cohen I, Navarro V, Clemenceau S, et al. On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science 2002; 298: 1418–21.

132   Huberfeld G, Wittner L, Clemenceau S, et al. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. J. Neurosci. 2007; 27: 9866–73.

133   Pathak HR, Weissinger F, Terunuma M, et al.Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. J Neurosci. 2007 Dec 19;27(51):14012-22.

134   Muldoon SF, Villette V, Tressard T, et al. GABAergic inhibition shapes interictal dynamics in awake epileptic mice. Brain 2015; 138: 2875–90.

135   Ellender TJ, Raimondo J V, Irkle A, et al. Excitatory effects of parvalbumin-expressing interneurons maintain hippocampal epileptiform activity via synchronous afterdischarges. J. Neurosci. 2014; 34: 15208–22.

136   Sessolo M, Marcon I, Bovetti S, et al. Parvalbumin-Positive Inhibitory Interneurons Oppose Propagation But Favor Generation of Focal Epileptiform Activity. J. Neurosci. 2015; 35: 9544–57.

137   Isokawa M. Preservation of dendrites with the presence of reorganized mossy fiber collaterals in hippocampal dentate granule cells in patients with temporal lobe epilepsy. Brain Res. 1997; 744: 339–43.

138   Avoli M, Olivier A. Bursting in human epileptogenic neocortex is depressed by an N-methyl-D-aspartate antagonist. Neurosci. Lett. 1987; 76: 249–54.

139   Isokawa M, Levesque MF. Increased NMDA responses and dendritic degeneration in human epileptic hippocampal neurons in slices. Neurosci. Lett. 1991; 132: 212–6.

140   Turner DA, Wheal H V. Excitatory synaptic potentials in kainic acid-denervated rat CA1 pyramidal neurons. J. Neurosci. 1991; 11: 2786–94.

141   Bernard C, Anderson A, Becker A, et al. Acquired dendritic channelopathy in temporal lobe epilepsy. Science 2004; 305: 532–5.

142   Beck H, Yaari Y. Plasticity of intrinsic neuronal properties in CNS disorders. Nat. Rev. Neurosci. 2008; 9: 357–69.

143   Wykes RC, Heeroma JH, Mantoan L, et al. Optogenetic and potassium channel gene therapy in a rodent model of focal neocortical epilepsy. Sci. Transl. Med. 2012; 4: 161ra152.

144   Jirsa VK, Stacey WC, Quilichini PP, et al. On the nature of seizure dynamics. Brain 2014; 137: 2210–30.

145   Proix T, Bartolomei F, Chauvel P, et al. Permittivity coupling across brain regions determines seizure recruitment in partial epilepsy. J. Neurosci. 2014; 34: 15009–21.

146   Galanopoulou AS, Pitkänen A, Buckmaster PS, et al. What should models model? What needs to be modeled? In: Animal Models of Seizures and Eplilepsy. Amsterdam: Elsevier; 2017. p. 1107-1119.

147   Margerison JH, Corsellis JA. Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. Brain 1966; 89: 499–530.

148   Bruton CJ, Stevens JR, Frith CD. Epilepsy, psychosis, and schizophrenia: clinical and neuropathologic correlations. Neurology 1994; 44: 34–42.

149   Karhunen H, Jolkkonen J, Sivenius J, et al. Epileptogenesis after experimental focal cerebral ischemia. Neurochem. Res. 2005; 30: 1529–42.

150   Engel J. Introduction to temporal lobe epilepsy. Epilepsy Res. 1996; 26: 141–50.

151   Borelli P, Shorvon SD, Stevens JM, et al. Extratemporal ictal clinical features in hippocampal sclerosis: their relationship to the degree of hippocampal volume loss and to the outcome of temporal lobectomy. Epilepsia 2008; 49: 1333–9.

152   Sloviter RS. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the 'dormant basket cell' hypothesis and its possible relevance to temporal lobe epilepsy. Hippocampus 1991; 1: 41–66.

153   Scharfman HE. Epileptogenesis in the parahippocampal region. Parallels with the dentate gyrus. Ann. N. Y. Acad. Sci. 2000; 911: 305–27.

154   Swartz BE, Houser CR, Tomiyasu U, et al. Hippocampal cell loss in posttraumatic human epilepsy. Epilepsia 2006; 47: 1373–82.

155   Pitkänen A, McIntosh TK. Animal models of post-traumatic epilepsy. J. Neurotrauma 2006; 23: 241–61.

156   Huusko N, Römer C, Ndode-Ekane XE, et al. Loss of hippocampal interneurons and epileptogenesis: a comparison of two animal models of acquired epilepsy. Brain Struct. Funct. 2013; 220: 153–91.

157   Diaz-Arrastia R, Agostini MA, Madden CJ, et al. Posttraumatic epilepsy: the endophenotypes of a human model of epileptogenesis. Epilepsia 2009; 50 Suppl 2: 14–20.

158   Brezova V, Moen KG, Skandsen T, et al. Prospective longitudinal MRI study of brain volumes and diffusion changes during the first year after moderate to severe traumatic brain injury. NeuroImage. Clin. 2014; 5: 128–40.

159   Zappone CA, Sloviter RS. Translamellar disinhibition in the rat hippocampal dentate gyrus after seizure-induced degeneration of vulnerable hilar neurons. J. Neurosci. 2004; 24: 853–64.

160   Jinde S, Zsiros V, Jiang Z, et al. Hilar mossy cell degeneration causes transient dentate granule cell hyperexcitability and impaired pattern separation. Neuron 2012; 76: 1189–200.

161   Winson J, Abzug C. Gating of neuronal transmission in the hippocampus: efficacy of transmission varies with behavioral state. Science 1977; 196: 1223–5.

162   Winson J, Abzug C. Neuronal transmission through hippocampal pathways dependent on behavior. J. Neurophysiol. 1978; 41: 716–32.

163   Heinemann U, Beck H, Dreier JP, et al. The dentate gyrus as a regulated gate for the propagation of epileptiform activity. Epilepsy Res. Suppl. 1992; 7: 273–

164   Lothman EW, Stringer JL, Bertram EH. The dentate gyrus as a control point for seizures in the hippocampus and beyond. Epilepsy Res. Suppl. 1992; 7: 301–13.

165   Sloviter RS. The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy. Ann. Neurol. 1994; 35: 640–54.

166   Lowenstein DH, Thomas MJ, Smith DH, et al. Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. J. Neurosci. 1992; 12: 4846–53.

167   Sloviter RS, Zappone CA, Harvey BD, et al. 'Dormant basket cell' hypothesis revisited: relative vulnerabilities of dentate gyrus mossy cells and inhibitory interneurons after hippocampal status epilepticus in the rat. J. Comp. Neurol. 2003; 459: 44–76.

168   Du F, Whetsell WO, Abou-Khalil B, et al. Preferential neuronal loss in layer III of the entorhinal cortex in patients with temporal lobe epilepsy. Epilepsy Res. 1993; 16: 223–33.

169   Pitkänen A, Engel J. Past and Present Definitions of Epileptogenesis and Its Biomarkers Neurotherapeutics 2014; 11: 231–241.

170   Wasterlain CG, Mazarati AM, Shirasaka Y, et al. Seizure-induced hippocampal damage and

chronic epilepsy: a hebbian theory of epileptogenesis. Adv. Neurol. 1999; 79: 829–43.

171   Dingledine R, Varvel NH, Dudek FE. When and how do seizures kill neurons, and is cell death relevant to epileptogenesis? Adv Exp Med Biol. 2014, 813:109-22.

172   Dingledine R, DA Coulter, B Fritsch, et al. (2017). Transcriptional profile of hippocampal dentate granule cells in four rat epilepsy models. Scientific Data, 4:170061.

173   Kahane P, Barba C, Rheims S, et al. The concept of temporal 'plus' epilepsy. Rev. Neurol. (Paris). 2015; 171: 267–72.

174   Amaral D, Lavenex P. Hippocampal neuroanatomy. In: The Hippocampus Book. New York: Oxford University Press; 2007. p. 872.

175   Babb TL, Halgren E, Wilson C, et al. Neuronal firing patterns during the spread of an occipital lobe seizure to the temporal lobes in man. Electroencephalogr. Clin. Neurophysiol. 1981; 51: 104–7.

176   Shihabuddin B, Abou-Khalil B, Delbeke D, et al. Orbito-frontal epilepsy masquerading as temporal lobe epilepsy-a case report. Seizure 2001; 10: 134–8.

177   Devinsky O, Vezzani A, Najjar S, et al. Glia and epilepsy: excitability and inflammation. Trends Neurosci. 2013; 36: 174–84.

178   Seifert G, Steinhäuser C. Neuron-astrocyte signaling and epilepsy. Exp. Neurol. 2013; 244: 4–10.

179   Vezzani A, Aronica E, Mazarati A, et al. Epilepsy and brain inflammation. Exp. Neurol. 2013a; 244: 11–21.

180   Boison D, Aronica E. Comorbidities in Neurology: Is adenosine the common link? Neuropharmacology 2015; 97: 18–34.

181   Farina C, Aloisi F, Meinl E. Astrocytes are active players in cerebral innate immunity. Trends Immunol. 2007; 28: 138–45.

182   Vezzani A, Ravizza T, Balosso S, et al. Glia as a source of cytokines: implications for neuronal excitability and survival. Epilepsia 2008; 49 Suppl 2: 24–32.

183   Wetherington J, Serrano G, Dingledine R. Astrocytes in the epileptic brain. Neuron. 2008, 58:168-78.

184   Aronica E, Ravizza T, Zurolo E, et al. Astrocyte immune responses in epilepsy. Glia 2012; 60: 1258–68.

185   Bedner P, Dupper A, Hüttmann K, et al. Astrocyte uncoupling as a cause of human temporal lobe epilepsy. Brain 2015; 138: 1208–22.

186   Khan D, Dupper A, Deshpande T, et al. Experimental febrile seizures impair interastrocytic

gap junction coupling in juvenile mice. J. Neurosci. Res. 2016; 94: 804–13.

187   Seifert G, Schilling K, Steinhäuser C. Astrocyte dysfunction in neurological disorders: a molecular perspective. Nat. Rev. Neurosci. 2006; 7: 194–206.

188   Seifert G, Carmignoto G, Steinhäuser C. Astrocyte dysfunction in epilepsy. Brain Res. Rev. 2010; 63: 212–21.

189   Steinhäuser C, Seifert G, Bedner P. Astrocyte dysfunction in temporal lobe epilepsy: K+ channels and gap junction coupling. Glia 2012; 60: 1192–202.

190   D'Ambrosio R, Maris DO, Grady MS, et al. Impaired K(+) homeostasis and altered electrophysiological properties of post-traumatic hippocampal glia. J. Neurosci. 1999; 19: 8152–62.

191   Köller H, Schroeter M, Jander S, et al. Time course of inwardly rectifying K(+) current reduction in glial cells surrounding ischemic brain lesions. Brain Res. 2000; 872: 194–8.

192   David Y, Cacheaux LP, Ivens S, et al. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? J. Neurosci. 2009; 29: 10588–99.

193   Schröder W, Hager G, Kouprijanova E, et al. Lesion-induced changes of electrophysiological properties in astrocytes of the rat dentate gyrus. Glia 1999; 28: 166–74.

194   Bordey A, Hablitz JJ, Sontheimer H. Reactive astrocytes show enhanced inwardly rectifying K+ currents in situ. Neuroreport 2000; 11: 3151–5.

195   Bordey A, Lyons SA, Hablitz JJ, et al. Electrophysiological characteristics of reactive astrocytes in experimental cortical dysplasia. J. Neurophysiol. 2001; 85: 1719–31.

196   Hinterkeuser S, Schröder W, Hager G, et al. Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. Eur. J. Neurosci. 2000; 12: 2087–96.

197   Cotrina ML, Kang J, Lin JH, et al. Astrocytic gap junctions remain open during ischemic conditions. J. Neurosci. 1998; 18: 2520–37

198   Ohsumi A, Nawashiro H, Otani N, et al. Temporal and spatial profile of phosphorylated connexin43 after traumatic brain injury in rats. J. Neurotrauma 2010; 27: 1255–63.

199   Bar-Klein G, Cacheaux LP, Kamintsky L, et al. Losartan prevents acquired epilepsy via TGF-β signaling suppression. Ann Neurol. 2014; 75:864-75.

200   Michel MC, Foster C, Brunner HR, et al. A systematic comparison of the properties of clinically used angiotensin II type 1 receptor antagonists. Pharmacol Rev. 2013; 65: 809-48.

201   Boison D. Adenosine kinase: exploitation for therapeutic gain. Pharmacol. Rev. 2013; 65: 906–43.

202   Gouder N, Scheurer L, Fritschy J-M, et al. Overexpression of adenosine kinase in epileptic hippocampus contributes to epileptogenesis. J. Neurosci. 2004; 24: 692–701.

203   Li T, Ren G, Lusardi T, et al. Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. J. Clin. Invest. 2008; 118: 571–82.

204   Pignataro G, Maysami S, Studer FE, et al. Downregulation of hippocampal adenosine kinase after focal ischemia as potential endogenous neuroprotective mechanism. J. Cereb. Blood Flow Metab. 2008; 28: 17–23.

205   Aronica E, Zurolo E, Iyer A, et al. Upregulation of adenosine kinase in astrocytes in experimental and human temporal lobe epilepsy. Epilepsia 2011; 52: 1645–55.

206   Li T, Lytle N, Lan J-Q, et al. Local disruption of glial adenosine homeostasis in mice associates with focal electrographic seizures: a first step in epileptogenesis? Glia 2012; 60: 83-95.

207   Lusardi TA, Lytle NK, Szybala C, et al. Caffeine prevents acute mortality after TBI in rats without increased morbidity. Exp. Neurol. 2012; 234: 161–8.

208   Masino SA, Li T, Theofilas P. A ketogenic diet suppresses seizures in mice through adenosine A_1 receptors. J. Clin. Invest. 2011; 121: 2679–83.

209   de Groot M, Iyer A, Zurolo E, et al. Overexpression of ADK in human astrocytic tumors and peritumoral tissue is related to tumor-associated epilepsy. Epilepsia 2012; 53: 58–66.

210   Aronica E, Sandau US, Iyer A, et al. Glial adenosine kinase--a neuropathological marker of the epileptic brain. Neurochem. Int. 2013; 63: 688–95.

211   Li T, Quan Lan J, Fredholm BB, et al. Adenosine dysfunction in astrogliosis: cause for seizure generation? Neuron Glia Biol. 2007; 3: 353–66.

212   Boison D, Huber A, Padrun V, et al. Seizure suppression by adenosine-releasing cells is independent of seizure frequency. Epilepsia 2002; 43: 788–96.

213   Fedele DE, Koch P, Scheurer L, et al. Engineering embryonic stem cell derived glia for adenosine delivery. Neurosci. Lett. 2004; 370: 160–5.

214   Lusardi TA, Akula KK, Coffman SQ, et al. Ketogenic diet prevents epileptogenesis and disease progression in adult mice and rats. Neuropharmacology 2015; 99: 500–9.

215   Eid T, Lee TS, Thomas MJ, et al. Loss of perivascular aquaporin 4 may underlie deficient water and K+ homeostasis in the humanepileptogenic hippocampus. Proc. Natl. Acad. Sci. U. S. A. 2005, 102, 1193–1198.

216   Strohschein S, Hüttmann K, Gabriel S, et al. Impact of aquaporin-4 channels on K+ buffering and gap junction coupling in the hippocampus. Glia 2011; 59: 973-980.

217   Binder DK, Nagelhus EA, Ottersen OP. Aquaporin-4 and epilepsy. Glia. 2012, 60:1203-14.

218   Hubbard JA, Szu JI, Yonan JM, et al. Regulation of astrocyte glutamate transporter-1 (GLT1) and aquaporin-4 (AQP4) expression in a model of epilepsy. Exp Neurol. 2016, 283(Pt A):85-96.

219   Filiou MD, Arefin AS, Moscato P, et al. 'Neuroinflammation' differs categorically from inflammation: transcriptomes of Alzheimer's disease, Parkinson's disease, schizophrenia and inflammatory diseases compared. Neurogenetics 2014; 15:201-212.

220   Kim JY, Kim N, Yenari MA. Mechanisms and potential therapeutic applications of microglial activation after brain injury. CNS Neurosci. Ther. 2015; 21:309-19.

221   Fabene PF, Bramanti P, Constantin G. The emerging role for chemokines in epilepsy. J. Neuroimmunol. 2010; 224: 22–7.

222   Friedman A, Bar-Klein G, Serlin Y, et al. Should losartan be administered following brain injury? Expert Rev. Neurother. 2014; 14: 1365–75.

223   Vezzani A, Viviani B. Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. Neuropharmacology 2015; 96: 70-82.

224   Aronica E, Crino P. Inflammation in Epilepsy: Clinical Observations. Epilepsia. 2011 May;52 Suppl 3:26-32

225   Dedeurwaerdere S1, Friedman A, Fabene PF, et al. Finding a better drug for epilepsy: antiinflammatory targets. Epilepsia. 2012 Jul;53(7):1113-8

226   Vezzani A, Fujinami RS, White HS, et al. Infections, inflammation and epilepsy. Acta Neuropathol. 2016; 131: 211–34.

227   Wraith DC, Nicholson LB. The adaptive immune system in diseases of the central nervous system. J. Clin. Inv. 2012; 122:1172-1179.

228   Chiu CC, Liao YE, Yang LY, et al. 2016. Neuroinflammation in animal models of traumatic brain injury. J. Neurosci. Methods 2016; 272:38-49.

229   Kim JY, Park JP, Chang JY, et al. Inflammation after Ischemic Stroke: The Role of Leukocytes and Glial Cells. Exp. Neurobiol. 2016; 25:241-251.

230   Iori V, Maroso M, Rizzi M, et al. Receptor for Advanced Glycation Endproducts is upregulated in temporal lobe epilepsy and contributes to experimental seizures. Neurobiol. Dis. 2013; 58:102-14.

231   Iori V, Iyer A, Ravizza T, et al. Blockade of the IL-1R1/TLR4 pathway mediates disease-modification therapeutic effects in a model of acquired epilepsy. Neurobiol. Dis. 2017; 99: 12-23.

232   Walker LE, Frigerio F, Ravizza T, et al. Molecular isoforms of HMGB1 are novel mechanistic biomarkers for epilepsy. J Clin. Invest. 2017; 127:2118–2132.

233   Libbey JE, Kennett NJ, Wilcox KS, et al. Interleukin-6, Produced by Resident Cells of the

Central Nervous System and Infiltrating Cells, Contributes to the Development of Seizures following Viral Infection. J. Virol. 2011; 85:6913-6922.

234   Kwon YS, Pineda E, Auvin S, et al. Neuroprotective and antiepileptogenic effects of combination of anti-inflammatory drugs in the immature brain. J. Neuroinflammation. 2013; 10: 30.

235   Dey A, Kang X, Qiu J, et al. Anti-Inflammatory Small Molecules To Treat Seizures and Epilepsy: From Bench to Bedside. Trends Pharmacol. Sci. 2016; 37:463-84.

236   Hunt RF, Boychuk JA, Smith BN. Neural circuit mechanisms of post-traumatic epilepsy. Front. Cell. Neurosci. 2013; 7:89

237   Zelano J. Poststroke epilepsy: update and future directions. Ther. Adv. Neurol. Disord. 2016; 9:424-35.

238   Russo M V, McGavern DB. Inflammatory neuroprotection following traumatic brain injury. Science 2016; 353: 783–5.

239   Sordillo PP, Sordillo LA, Helson L. Bifunctional role of pro-inflammatory cytokines after traumatic brain injury. Brain Inj. 2016; 30: 1043–53.

240   Febinger HY, Thomasy HE, Pavlova MN, et al. Time-dependent effects of CX3CR1 in a mouse model of mild traumatic brain injury. J. Neuroinflamm. 2015;12:154.

241   Scherbel U, Raghupathi R, Nakamura M, et al. Differential acute and chronic responses of tumor necrosis factor-deficient mice to experimental brain injury. Proc. Natl. Acad. Sci. U S A. 1999; 96: 8721-6.

242   Zanier ER, Marchesi F, Ortolano F, et al. Fractalkine Receptor Deficiency Is Associated with Early Protection but Late Worsening of Outcome following Brain Trauma in Mice. J. Neurotrauma. 2016; 33:1060-72

243   Chamorro Á, Meisel A, Planas AM, et al. The immunology of acute stroke. Nat. Rev. Neurol. 2012; 8: 401–10.

244   Clausen BH, Degn M, Sivasaravanaparan M, et al. Conditional ablation of myeloid TNF increases lesion volume after experimental stroke in mice, possibly via altered ERK1/2 signaling. Sci. Rep. 2016; 6: 29291.

245   Mracsko E, Veltkamp R. Neuroinflammation after intracerebral hemorrhage. Front. Cell. Neurosci. 2014; 8: 388.

246   Parker TM, Nguyen AH, Rabang JR, et al. The danger zone: Systematic review of the role of HMGB1 danger signalling in traumatic brain injury. Brain Inj. 2017; 31: 2–8.

247   Pauletti A, Terrone G, Ahmad-hekh T, et al. Targeting oxidative stress improves disease outcomes in a rat model of acquired epilepsy. Brain 2017; 140:1885-1899.

248   Zhao J, Wang Y, Xu C, et al. Therapeutic potential of an anti-high mobility group box-1 monoclonal antibody in epilepsy. Brain Behav Immun. 2017 Feb 3. pii: S0889-1591(17)30024-7. doi: 10.1016/j.bbi.2017.02.002. [Epub ahead of print].

249   Carty M, Reinert L, Paludan SR, et al. Innate antiviral signalling in the central nervous system. Trends Immunol. 2014; 35: 79–87.

250   Lund IV, Hu Y, Raol YH, et al. BDNF selectively regulates GABAA receptor transcription by activation of the JAK/STAT pathway. Sci. Signal. 2008; 1 (41):ra9.

251   Oliva AA, Kang Y, Sanchez-Molano J, et al. STAT3 signaling after traumatic brain injury. J. Neurochem. 2012; 120: 710–20.

252   Raible DJ, Frey LC, Cruz Del Angel Y, et al. GABA(A) receptor regulation after experimental traumatic brain injury. J. Neurotrauma 2012; 29: 2548–54.

253   Satriotomo I, Bowen KK, Vemuganti R. JAK2 and STAT3 activation contributes to neuronal damage following transient focal cerebral ischemia. J. Neurochem. 2006; 98: 1353–68.

254   Suzuki S, Tanaka K, Nogawa S, et al. Phosphorylation of signal transducer and activator of transcription-3 (Stat3) after focal cerebral ischemia in rats. Exp. Neurol. 2001; 170: 63–71.

255   Zhao J, Zhang Y, Li G, et al. Activation of JAK2/STAT pathway in cerebral cortex after experimental traumatic brain injury of rats. Neurosci. Lett. 2011; 498: 147–52.

256   Grabenstatter HL, Cruz Del Angel Y, Carlsen J, et al. The effect of STAT3 inhibition on status epilepticus and subsequent spontaneous seizures in the pilocarpine model of acquired epilepsy. Neurobiol. Dis. 2014; 62: 73–85.

257   Raible DJ, Frey LC, Cruz Del Angel Y, et al. JAK/STAT pathway regulation of GABAA receptor expression after differing severities of experimental TBI. Exp. Neurol. 2015; 271: 445–56.

258   Brewster AL, Lugo JN, Patil V V, et al. Rapamycin reverses status epilepticus-induced memory deficits and dendritic damage. PLoS One 2013; 8: e57808.

259   Guo D, Zeng L, Brody DL, Wong M. Rapamycin attenuates the development of posttraumatic epilepsy in a mouse model of traumatic brain injury. PLoS One 2013; 8: e64078.

260   Zeng L-H, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. J. Neurosci. 2009; 29: 6964–72.

261   Galanopoulou AS, Gorter JA, Cepeda C. Finding a better drug for epilepsy: the mTOR pathway as an antiepileptogenic target. Epilepsia 2012; 53: 1119–30.

262   Huang X, Zhang H, Yang J, et al. Pharmacological inhibition of the mammalian target of rapamycin pathway suppresses acquired epilepsy. Neurobiol Dis. 2010, 40(1):193-9.

263   Butler CR, Boychuk JA, Smith BN. Effects of Rapamycin Treatment on Neurogenesis and

Synaptic Reorganization in the Dentate Gyrus after Controlled Cortical Impact Injury in Mice. Front. Syst. Neurosci. 2015; 9

264   Drion CM, Borm LE, Kooijman L, et al. Effects of rapamycin and curcumin treatment on the development of epilepsy after electrically induced status epilepticus in rats. Epilepsia 2016; 57: 688–697.

265   McDaniel SS, Wong M. Therapeutic role of mammalian target of rapamycin (mTOR) inhibition in preventing epileptogenesis. Neurosci. Lett. 2011; 497: 231–239.

266   Raffo E, Coppola A, Ono T, et al. A pulse rapamycin therapy for infantile spasms and associated cognitive decline. Neurobiol. Dis. 2011; 43: 322–9.

267   Rensing N, Han L, Wong M. Intermittent dosing of rapamycin maintains antiepileptogenic effects in a mouse model of tuberous sclerosis complex. Epilepsia 2015; 56: 1088–1097.

268   Boulle F, Kenis G, Cazorla M, et al. TrkB inhibition as a therapeutic target for CNS-related disorders. Prog Neurobiol 2012; 98: 197-206.

269   Gu B, Huang YZ, He X-P, et al. A Peptide Uncoupling BDNF Receptor TrkB from Phospholipase Cγ1 Prevents Epilepsy Induced by Status Epilepticus. Neuron 2015; 88: 484–91.

270   Vezzani A, Maroso M, Balosso S, et al. IL-1 receptor/Toll-like receptor signaling in infection, inflammation, stress and neurodegeneration couples hyperexcitability and seizures. Brain Behav Immun 2011; 25: 1281-9.

271   Ravizza T, Noe F, Zardoni D, et al. Interleukin Converting Enzyme inhibition impairs kindling epileptogenesis in rats by blocking astrocytic IL-1beta production. Neurobiol Dis 2008; 31: 327-33.

272   Bertram E. The relevance of kindling for human epilepsy. Epilepsia 2007; 48 Suppl 2: 65–74.

273   Gorter JA, van Vliet EA, Lopes da Silva FH. Which insights have we gained from the kindling and post-status epilepticus models? J. Neurosci. Methods 2016; 260: 96–108.

274   Pitkänen A, Halonen T. Prevention of epilepsy. Trends Pharmacol. Sci. 1998; 19: 253–5.

275   Löscher W. Fit for purpose application of currently existing animal models in the discovery of novel epilepsy therapies. Epilepsy Res. 2016; 126: 157–84.

276   Löscher W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. Epilepsy Res. 2002; 50: 105–23.

277   Eastman CL, Verley DR, Fender JS, et al. ECoG studies of valproate, carbamazepine and halothane in frontal-lobe epilepsy induced by head injury in the rat. Exp. Neurol. 2010; 224: 369–88.

278   Eastman CL, Verley DR, Fender JS, et al. Antiepileptic and antiepileptogenic performance of

carisbamate after head injury in the rat: blind and randomized studies. J. Pharmacol. Exp. Ther. 2011; 336: 779–90.

279   Barker-Haliski ML, Dahle EJ, Heck TD, et al. Evaluating an etiologically relevant platform for therapy development for temporal lobe epilepsy: effects of carbamazepine and valproic acid on acute seizures and chronic behavioral comorbidities in the Theiler's murine encephalomyelitis virus mouse model. J. Pharmacol. Exp. Ther. 2015; 353: 318–29.

280   Silver JM, Shin C, McNamara JO. Antiepileptogenic effects of conventional anticonvulsants in the kindling model of epilespy. Ann. Neurol. 1991; 29: 356–63.

281   Löscher W, Hönack D, Rundfeldt C. Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. J. Pharmacol. Exp. Ther. 1998; 284: 474–9.

282   Kaminski RM, Rogawski MA, Klitgaard H. The potential of antiseizure drugs and agents that act on novel molecular targets as antiepileptogenic treatments. Neurotherapeutics 2014; 11: 385–400.

283   Pitkänen A, Nehlig A, Brooks-Kayal AR, et al. Issues related to development of antiepileptogenic therapies. Epilepsia 2013; 54 Suppl 4: 35–43.

284   Rojas A, Jiang J, Ganesh T, et al. Cyclooxygenase-2 in epilepsy. Epilepsia. 2014, 55:17-25.

285   Kaila K, Price TJ, Payne JA, et al. Cation-chloride cotransporters in neuronal development, plasticity and disease. Nat. Rev. Neurosci. 2014; 15: 637–54.

286   Brandt C, Nozadze M, Heuchert N, et al. Disease-modifying effects of phenobarbital and the NKCC1 inhibitor bumetanide in the pilocarpine model of temporal lobe epilepsy. J. Neurosci. 2010; 30: 8602–12.

287   Koyama R, Tao K, Sasaki T, et al. GABAergic excitation after febrile seizures induces ectopic granule cells and adult epilepsy. Nat. Med. 2012; 18: 1271–8.

288   Marguet SL, Le-Schulte VTQ, Merseburg A, et al. Treatment during a vulnerable developmental period rescues a genetic epilepsy. Nat. Med. 2015; 21: 1436–44.

289   Bar-Klein G, Klee R, Brandt C, et al. Isoflurane prevents acquired epilepsy in rat models of temporal lobe epilepsy. Ann Neurol 2016; 80: 896-908.

290   Scicchitano F, Constanti A, Citraro R, et al. Statins and epilepsy: preclinical studies, clinical trials and statin-anticonvulsant drug interactions. Curr. Drug Targets 2015; 16: 747–56.

291   Barker-Haliski ML, Heck TD, Dahle EJ, et al. Acute treatment with minocycline, but not valproic acid, improves long-term behavioral outcomes in the Theiler's virus model of temporal lobe epilepsy. Epilepsia 2016; 57: 1958–1967.

292   Siniscalchi A, Mintzer S. Statins for poststroke seizures: the first antiepileptogenic agent?

Neurology. 2015; 85:661-2.

293   Pugh MJ V, Knoefel JE, Mortensen EM, et al. New-onset epilepsy risk factors in older veterans. J. Am. Geriatr. Soc. 2009; 57: 237–42.

294   Etminan M, Samii A, Brophy JM. Statin use and risk of epilepsy: a nested case-control study. Neurology 2010; 75: 1496–500.

295   White HS, Löscher W. Searching for the ideal antiepileptogenic agent in experimental models: single treatment versus combinatorial treatment strategies. Neurotherapeutics. 2014 Apr;11(2):373-84

296   Johnson MR, Shkura K, Langley SR, et al. Systems genetics identifies a convergent gene network for cognition and neurodevelopmental disease. Nat Neurosci. 2016, 19:223-32.

297   Delahaye-Duriez A, Srivastava P, Shkura K, et al. Rare and common epilepsies converge on a shared gene regulatory network providing opportunities for novel antiepileptic drug discovery. Genome Biol. 2016. 17:245.

298   Bassett DS, Sporns O. Network neuroscience. Nat Neurosci. 2017, 20:353-364.

299   Pangalos MN, Schechter LE, Hurko O. Drug development for CNS disorders: strategies for balancing risk and reducing attrition. Nat. Rev. Drug Discov. 2007; 6: 521–32.

300   Gershen LD, Zanotti-Fregonara P, Dustin IH, et al. Neuroinflammation in Temporal Lobe Epilepsy Measured Using Positron Emission Tomographic Imaging of Translocator Protein. JAMA Neurol. 2015; 72: 882–8.

301   Finnema SJ, Nabulsi NB, Eid T, et al. Imaging synaptic density in the living human brain. Sci. Transl. Med. 2016; 8: 348ra96.

302   Falco-Walter J, Owen C, Sharma M, et al. Magnetoencephalography and New Imaging Modalities in Epilepsy. Neurotherapeutics 2017; 14: 4–10.

303   Thomas DW, Burns J, Audette J, et al. Clinical Development Success Rates 2006-2015, Bioindustry Analysis Report, June 2016.

304   McGhee DJM, Ritchie CW, Zajicek JP, et al. A review of clinical trial designs used to detect a disease-modifying effect of drug therapy in Alzheimer's disease and Parkinson's disease. BMC Neurol. 2016; 16: 92.

305   Schmidt D, Friedman D, Dichter MA. Anti-epileptogenic clinical trial designs in epilepsy: issues and options. Neurotherapeutics. 2014; 11: 401-11.

306   U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for Industry Codevelopment of Two or More New Investigational Drugs for Use in Combination. June 2013: https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm236669.pdf

307   U.S. Department of Health and Human Services, Food and Drug Administration, Office of the Commissioner, Office of Combination Products. Guidance for Industry and FDA Staff: Early Development Considerations for Innovative Combination Products. September 2006: https://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm126054.pdf

308   Racine RJ, Paxinos G, Mosher JM, et al. The effects of various lesions and knife-cuts on septal and amygdala kindling in the rat. Brain Res. 1988; 454: 264–74.

309   Meeren HKM, Pijn JPM, Van Luijtelaar ELJM, et al. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. J. Neurosci. 2002; 22: 1480–95.

310   Braganza O, Bedner P, Hüttmann K, et al. Albumin is taken up by hippocampal NG2 cells and astrocytes and decreases gap junction coupling. Epilepsia 2012; 53: 1898-1906.

311   Friedman A, Heinemann U. Role of Blood-Brain Barrier Dysfunction in Epileptogenesis. In: Noebels JL, Avoli M, Rogawski MA, et al. Jasper's Basic Mechanisms of the Epilepsies, 4th ed. Bethesda (MD): National Center for Biotechnology Information (US); 2012. p. 1-12.

312   Majkowski J, Bilińska-Nigot B, Sobieszek A. Development of EEG epileptic activity and seizures during kindling in sensorimotor cortex in cats. Epilepsia 1981; 22: 275–84.

313   Bolkvadze T, Pitkänen A. Development of post-traumatic epilepsy after controlled cortical impact and lateral fluid-percussion-induced brain injury in the mouse. J. Neurotrauma 2012; 29: 789–812.

314   Kelly KM, Miller ER, Lepsveridze E, et al. Posttraumatic seizures and epilepsy in adult rats after controlled cortical impact. Epilepsy Res. 2015; 117: 104–16.

315   Piredda S, Gale K. A crucial epileptogenic site in the deep prepiriform cortex. Nature; 317: 623–5.

316   Cavalheiro EA, Naffah-Mazzacoratti MG, Mello LE, et al. The Pilocarpine Model of Seizures In: Models of Seizures and Epilepsy. Elsevier; 2006. p. 433–448.

317   Goddard G V. Development of Epileptic Seizures through Brain Stimulation at Low Intensity Nature 1967; 214: 1020–1021.

318   Olney JW, Rhee V, Ho OL. Kainic acid: a powerful neurotoxic analogue of glutamate. Brain Res. 1974; 77: 507–12.A

319   Racine R, Rose PA, Burnham WM. Afterdischarge thresholds and kindling rates in dorsal and ventral hippocampus and dentate gyrus. Can. J. Neurol. Sci. 1977; 4: 273–8.

320   Weissberg I, Wood L, Kamintsky L, et al. Albumin induces excitatory synaptogenesis through astrocytic TGF-beta/ALK5 signaling in a model of acquired epilepsy following blood-brain barrier dysfunction. Neurobiol Dis 2015; 78: 115-25.

321   Englander J, Bushnik T, Duong TT, et al. Analyzing risk factors for late posttraumatic seizures: a prospective, multicenter investigation. Arch. Phys. Med. Rehabil. 2003; 84: 365-73.

322   Annegers JF, Hauser WA, Coan SP, et al. A population-based study of seizures after traumatic brain injuries. N. Engl. J. Med. 1998; 338: 20-4.

323   Graham NSN, Crichton S, Koutroumanidis M, et al. Incidence and Associations of Poststroke Epilepsy: The Prospective South London Stroke Register. Stroke 2013; 44: 605-611.

324   Annegers JF, Hauser WA, Beghi E, et al. The risk of unprovoked seizures after encephalitis and meningitis. Neurology 1988; 38: 1407-10.

325   Haapaniemi E, Strbian D, Rossi C, et al. The CAVE Score for Predicting Late Seizures After Intracerebral Hemorrhage. Stroke 2014; 45: 1971-1976.

Supporting Information

Additional Supporting Information may be found in the online version of this article

Legends to the figures

Fig. 1

Epileptogenic processes and risk factors involved in development of epilepsy after acute brain insults: a conceptual view. Possibly depending on crucial modifiers or risk factors, the same brain injury can be epileptogenic or not. In the majority of patients, brain insults do not cause epilepsy as discussed in the text. Furthermore, as illustrated in the figure, not all epileptogenic processes, once initiated, result in epilepsy, i.e. complete their course to clinically obvious disease. The term epileptogenesis includes processes that render the brain susceptible to spontaneous recurrent seizures <u>and</u> processes that intensify seizures and make them more refractory to therapy (progression). During epileptogenesis, multiple brain alterations occur, including altered excitability of neurons and/or neuronal circuits, activation of microglia, astrocyte dysfunction, alterations in expression and function of receptors and ion channels (in part recapitulating ontogenesis), loss of neurons, neurogenesis, axonal and dendritic sprouting, gliosis, inflammatory processes, and more. It is important to note that some of these alterations may be related to post-injury repair or recovery and not suited as targets to halt the epileptogenic process. The figure has been modified from previous versions.[3,15,276]

Fig. 2

Common patterns of astrogliosis in epileptic human brain tissue. *A:* Reactive astrogliosis in the neocortex of human epilepsy surgery brain specimens is commonly seen along cortical capillaries (arrow). *B:* In white matter, there is another common pattern of astrogliosis built by a dense glial fibrillary meshwork. The arrow points towards an enlarged venous vessel. Scale bar in A = 100 µm, in B = 200 µm. GFAP immunohistochemistry (brownish color) with bluish hematoxylin counterstaining.

Fig. 3

Common patterns of heterotopic neurons in the white matter of epileptic human brain tissue. *A:* The white matter of human temporal lobe usually contains only single heterotopic neurons. *B:* In many epilepsy specimens of human temporal lobe, there is a vast excess of heterotopic neurons with ramifying neuronal processes in white matter. Scale bar in A and B = 200 µm. MAP2 immunohistochemistry (brownish color) with bluish hematoxylin counterstaining.

Fig. 4

Schematic illustrating components of the limbic (A) and thalamocortical (B) seizure circuits. Areas impacted by particular epileptogenic insults (both classic and newer models of acquired epilepsy) are noted near particular network nodes. Arrows indicate interconnections between the nodes. Due to the distributed nature of these circuits, many sites may trigger, lead, or participate in seizure activity.[308,309]

Fig. 5

Word clouds summarizing altered biological processes in the hippocampus in experimental models of structural epilepsies and in human drug-refractory temporal lobe epilepsy (TLE). The analysis included **(A)** genome-wide (GW) changes in gene expression and **(B)** predicted targets of regulated miRNAs. Studies included in the analysis are listed in Supplementary Tables 2 and 3. Biological functions/processes were collected from the gene ontology (GO) or pathway analyses performed in each study. The data was organized to align with different phases of epileptogenic process (acute post-injury time point, latency phase, and epilepsy phase). Word clouds were generated using Wordcloud for R Package. For the sake of clarity, the words: 'regulation', 'cell', 'cells', 'process' and stop words

('English') were removed from the figures. All other words presented in original articles were included (minimum frequency=1). Larger the word font size in the cloud, more often the word appeared *e.g.* in the DAVID or IPA® analyses. Note that there were differences in the original analyses, for example, in the terminology used to name different classes of biological functions and use of cut-off p-values (*e.g.,* analyses performed by DAVID or IPA®). Also, the word cloud generated from the human data in panel B is not unbiased (asterisk) as it was extracted from the text (no pathway or GO analysis was presented by authors). Major differences between models were observed, especially when predicted miRNA-target regulated processes were compared **(B)**. SE models appeared more similar with each other than with TBI.

Fig. 6

Pathophysiological immune/inflammatory sequelae triggered by various acute brain injuries. Rapid activation of brain resident innate immunity cells at the site of injury and BBB functional and structural alterations result in the generation of the inflammatory cascade in seizure-prone brain areas. Leukocytes may contribute to perpetuate the inflammatory cascade following interactions with the brain vasculature. The time-locked sequence of these events likely depends on the type of injury. Inflammatory mediators can promote brain damage and dysfunction or contribute to tissue repair depending on their levels and persistence. If the inflammatory *milieu* exceeds the homeostatic threshold it may lead to hyperexcitability, decrease seizure threshold and promote neuropathology thereby contributing to disease progression. These pathologic events depend on the ability of inflammatory molecules such as cytokines, DAMPs and prostaglandins to alter expression and function of gap junctions, voltage-gated or receptor-coupled ion channels thus contributing to acquired channelopathies, to modify GABA and glutamate release and re-uptake [176,177,185] and to alter BBB permeability. BBB dysfunction results in albumin

extravasation which compromises homeostatic astrocytic functions and induces excitatory synaptogenesis by activating the TGF-β/ALK5 signaling.[310,311,320]

Fig. 7

Signaling pathway commonalities. Epileptogenic brain injuries, including TBI, stroke, and SE, increase levels of growth factors, cytokines and hormones that activate the (A) JAK/STAT, IL-1R1/TLR4 and mTORC1 signaling and (B) BDNF/trkB/PLCγ signaling pathways resulting in altered expression of genes and proteins involved in cell growth, cell survival, cell proliferation, neurotransmission, learning and memory, potentially contributing to epileptogenesis and cognitive co-morbidities.