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Animal models and human tissue compared to better understand and treat the epilepsies

Giampaolo Milior1 | Mélanie Morin-Brureau2 | Johan Pallud3,4,5 | Richard Miles1 | Gilles Huberfeld1,6

Abstract
Animal models of human brain disorders permit researchers to explore disease mechanisms and to test potential therapies. However, therapeutic molecules derived from animal models often translate poorly to the clinic. Although human data may be more relevant, experiments on patients are constrained, and living tissue is unavailable for many disorders. Here, we compare work on animal models and on human tissue for three epileptic syndromes where human tissue is excised therapeutically: (1) acquired temporal lobe epilepsies, (2) inherited epilepsies associated with cortical malformations, and (3) peritumoral epilepsies. Animal models rest on assumed equivalencies between human brains and brains of mice, the most frequently used model animal. We ask how differences between mouse and human brains could influence models. General principles and compromises in model construction and validation are examined for a range of neurological diseases. Models may be judged on how well they predict novel therapeutic molecules or new mechanisms. The efficacy and safety of new molecules are evaluated in clinical trials. We judge new mechanisms by comparing data from work on animal models with data from work on patient tissue. In conclusion, we stress the need to cross-verify findings from animal models and from living human tissue to avoid the assumption that mechanisms are identical.

KEYWORDS
animal model, antiseizure drugs, focal cortical dysplasia (FCD), human tissue, mechanisms, mesial temporal lobe epilepsy (MTLE), tumor-related epilepsy
1 INTRODUCTION

Robert Burns, the Scottish poet, wrote "The best laid schemes o' mice an' men, gang aft agley." It could be interpreted as "it may be a mistake to assume that humans and mice are similar." Here, we ask whether the same idea can be applied to neurological diseases with a focus on epileptic conditions for which both animal models and living human tissue have been studied. Of course, models of brain conditions have been constructed from many animal species other than mice, and humans including both women and men suffer from the epilepsies.

Animal models of human brain diseases, including the epilepsies, stroke, multiple sclerosis, and Alzheimer disease, have been hugely influential. They permit experiments that cannot be done in patients, tests of new treatments, and exploration of the mechanisms underlying neurological symptoms. Some animal strains replicate human diseases, but many human conditions have no direct animal analogue. Instead, models are constructed by reproducing genetic, biochemical, or other defects defined from imaging, anatomical, and molecular data from patients.

Most animal models have been based on mice, which are easy to maintain and amenable to genetic technologies that produce reproducible strains of homogenous animals. A mouse brain is clearly not a human brain. At the gross anatomical level, the volume of a mouse brain is about 2 cm³ compared to about 1400 cm³ for a human brain. Do similarities between rodent and human brains suffice to construct realistic mouse models of human neurological conditions?

At a molecular level, most proteins present in humans are also found in mice. However, approximately 10% (1947/18 713) of human genes have no mouse homologue, and approximately 12% of mouse genes (2266/19 032) have no human homologue. These data are based on sequence similarity and phenotype (Homologene, https://www.ncbi.nlm.nih.gov/homologene/). Precise amino acid sequences of homologue proteins may differ and could affect function. Mouse and human carbohydrate molecules seem to be similar, whereas the human lipidome is more diverse than that of the mouse.

Cell types of the brain are broadly similar in mice and humans, although human-specific types of astrocyte and cortical inhibitory interneuron have been suggested. Transcriptomic analysis suggests that cortical neuronal types are broadly conserved between mouse and human, with more subtypes of inhibitory than excitatory cells. Transcriptomic analysis of single, cortical microglia revealed comparable, if subtly different, signatures for human and mouse cells. The number of cells is much higher in the human, with ~92 billion neurons, than in the mouse, with ~71 million neurons.

Mouse lifetimes are significantly shorter, approximately 8% of a human lifetime. A shorter lifetime may be important for models of conditions such as age-dependent neuronal degeneration. Factors specific to the life of laboratory animals may influence models of neurological conditions. Laboratory mice raised in specific pathogen-free conditions possess inexperienced, immature immune systems. Comorbidities, such as hypertension in stroke, should be engineered in laboratory animals to obtain realistic models of some syndromes. Restricted social and environmental experiences may also affect brain disorders. For instance, seizure frequency is 10–20 times higher in singly housed mice treated to induce epilepsy, than in animals kept in groups. Thus, the genetic, environmental, and social homogeneity of laboratory mice may have both advantages and disadvantages in models of brain disorders.

Comparison of animal models with data from patients with the same condition would help assess whether similarities between rodent and human brains are sufficient. For many human neurological conditions, such data are limited to noninvasive tests on patients or postmortem analyses. However, human tissue is excised therapeutically from patients with some epilepsies and brain tumors. In living tissue, responses to stimuli can be measured with electrophysiology and detection of released molecules. In this review, we examine general principles for the construction and validation of animal models of neurological diseases. We compare data on new mechanisms emerging from work on living human tissue with those derived from animal models of the same epileptic disorder and examine the development of novel antiseizure drugs. The review concludes by stressing the importance of cross-validation of findings derived from work on living human tissue and from work on animal models.
VALIDATION OF ANIMAL MODELS OF BRAIN DISORDERS

Criteria for the validation of animal models of brain disorders were initially suggested for models of depression.\textsuperscript{19,20} Figure 1 illustrates the criteria schematically: (1) construct validity: is the model based on known causes of the human disease? (2) face validity: do symptoms in model animals reflect those of the human syndrome? and (3) predictive validity: Does the model predict unknown features of the human disease?

Construct validity (Figure 1A) is most convincingly satisfied when a model aims to replicate tangible features with a known basis and brain localization. For instance, mechanical shocks induce traumatic brain injuries in animals with primary and secondary symptoms comparable to those observed in human brain injuries.\textsuperscript{21} In contrast, modeling some psychiatric disorders may be more difficult, as a molecular basis is not always simple, biomarkers do not necessarily exist, and involved brain regions may not be clear\textsuperscript{22} (but see Walsh et al.\textsuperscript{23}). Models of a given syndrome are often improved as knowledge advances. Mouse models of Alzheimer disease, for instance, have been based on expression of several iterations of mutations defined in humans.\textsuperscript{24,25}

Face validity (Figure 1B) is best satisfied when symptoms in the animal model are unambiguous, easy to measure, and not too numerous. Thus, animal models of migraine face the difficulty that it is hard to measure chronic pain in rodents.\textsuperscript{26} For some complex human conditions, useful insights have been obtained by modeling different facets of the disease separately. In dysmyelinating disease, inflammatory aspects have been mimicked as experimental allergic encephalomyelitis induced by treatment with myelin-related antigens,\textsuperscript{3} whereas oligodendrocyte death and axonal demyelination have been induced using cuprizone.\textsuperscript{27} The

\begin{figure}
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\caption{Construct, face, and predictive validity in animal models, shown schematically for models of acquired focal epilepsies induced by the neurotoxin kainic acid (KA). (A) Construct validity. Intrahippocampal injection of neurotoxin is shown. Green represents staining of neuronal cell bodies with an antibody to the neuronal nuclear antigen (NeuN), and red represents a tracer (Fluoro-Ruby) injected with KA.\textsuperscript{160} KA-induced status epilepticus extracellular recordings from dorsally and ventrally injected ipsilateral hippocampus (ID, IV) and dorsal and ventral contralateral hippocampus (CD, CV) at 5 h after injection are shown.\textsuperscript{160} (B) Face validity. (Top) Symptoms include stereotyped patterns of pyramidal cell death in the hilus and the CA3 and CA1 regions. Cresyl violet stain of neuronal somata at 7 days after KA injection is shown (reproduced with permission from Cossart et al.\textsuperscript{42}). (Middle) Recurrent seizures occur spontaneously. Field records from ID, IV, CD, and CV at 4 months after KA.\textsuperscript{160} (Bottom) Seizures emerge after a delay. Seizure frequency is plotted against time after injection. Average frequency is shown in red. Data from three of nine recorded animals are shown in green, blue, and yellow (adapted with permission from Williams et al.\textsuperscript{50}). (C) Predictive validity for therapies and treatments, including the identification of key sites and new molecules, for mechanisms involving specific cell types and inter- or intracellular signaling.}
\end{figure}
multiple symptoms of Alzheimer disease, which include cognitive decline, neuronal loss, extracellular plaques, and neurofibrillary tangles, have not all been captured in a single animal model.

Predictive validity of a model (Figure 1C) may be judged by the metrics of therapies and mechanisms. Identification of novel biomarkers would also facilitate assessment of treatment effects. We will compare here new therapies and novel mechanisms derived from work on human tissue and from animal models of the same epileptic syndromes. However, progress from preclinical tests to successful clinical trials of new molecular entities has sometimes been difficult. Fewer than 1% of drugs proposed for Alzheimer disease have passed clinical trials, and drugs designed to alleviate neuronal degeneration after trauma have a similar poor record. Can these statistics be improved?

Multiple factors may thwart effective translation to a successful clinical trial. They include unexpected side effects in humans, distinct pharmacokinetics, and dynamics and differences between animal and human physiology. Distinct procedures, such as different anesthetic protocols and timing of drug delivery in animal models, have invalidated some comparisons. Young, healthy laboratory animals are not useful when comorbidities contribute significantly to human diseases as for high blood pressure or diabetes in stroke patients. An improved alignment of animal and human protocols and more rigorous judgments on outcomes in initial stages of clinical trials could eliminate some difficulties.

3 | ANIMAL MODELS OF EPILEPTIC LESIONS AND SYNDROMES

There are many human epileptic conditions, with the common element of a local or globally synchronous neuronal firing. Some conditions develop after a shock or insult, whereas others have a genetic background. We will examine animal models of epileptic conditions for which work has also been done on living human tissue after therapeutic excision.

3.1 | Models of acquired temporal lobe epilepsy

Mesial temporal lobe epilepsy (MTLE) is often, but not always, acquired after a trigger event or injury that induces a seizure or status epilepticus. They include fever, trauma, or brain infection. Recurrent seizures initiated in the temporal lobe emerge in patients after a long delay of several years, required to establish epileptogenic remodeling of the brain.

Acquired temporal lobe epilepsies have been constructed in rodents using excitatory neurotoxins such as kainic acid (KA), pilocarpine, or electrical kindling. Treatment with neurotoxins induces a prolonged status epilepticus, with synchronous electroencephalographic (EEG) discharges and convulsive behaviors (Figure 1A). Two approaches have been used: systemic application by intraperitoneal injection or local injection in the hippocampus or regions projecting to it such as the amygdala or neocortex. One significant difference is that intraperitoneal injection induces wide-ranging and bilateral neuronal death, whereas local injection causes unilateral damage closer to patterns of cell death in patients. Features of an initial status epilepticus are mostly shared between intraperitoneal and focal injection, although animal mortality is higher with intraperitoneal injections.

The face validity of these models is satisfied in that mice injected with neurotoxins generate spontaneous, recurring seizures after a delay. Electrical and behavioral features of seizures, which are initiated in the hippocampal formation and spread to involve wider brain regions, are comparable to those of MTLE patients.

Multiple features of models of acquired epilepsies mimic data from MTLE patients, even if a single factor that underlies the emergence of an epileptic brain or initiates a seizure remains to be identified. Patterns of hippocampal pyramidal cell death in rodents and patients are comparable to those in patients. Some types of inhibitory cell die, and glial cells are activated. Axonal sprouting, when target cells are lost, leads to the growth of novel excitatory synapses sometimes contacting previously noninnervated targets.

In rodents, recurrent seizures begin at 2–5 weeks after neurotoxin treatment (Figure 1B). The delay before the onset of seizures is several years in the human. Neuronal death in mice occurs over days or weeks. In the human, the kinetics of neuronal death are slower as may be epileptogenic processes such as the formation of novel synapses. Possibly such processes are triggered by a single seizure and accelerated by complex status epilepticus. Radiological signs of hippocampal neuronal death at a delay of 12 months are greater after complex febrile seizures than single febrile convulsions.

3.2 | Models of epilepsies generated in tissue surrounding cerebral tumors

Cortical gliomas are highly epileptogenic tumors. Glioma cells proliferate and migrate into cortical regions around the tumor, and seizures are initiated from these peritumoral regions. Bidirectional signaling involving...
both γ-aminobutyric acid (GABA) and glutamate, from glioblastoma cells to the cortex and from cortical neurons to the tumor, is crucial for both seizures and tumor progression. In glioma cells, an upregulated potassium–chloride cotransporter loads internal Cl− to levels near 100 mmol·L−1, initiating water and Cl− fluxes that facilitate migration and proliferation.58 In peritumoral neurons, cotransporter dysregulation also increases Cl− levels and reduces GABA-mediated inhibition.59 In glioma cells, glutamate transport is compromised by disruption of Na-glutamate transporters and hyperactivation of the system xc− cysteine/glutamate exchanger.60,61

Work on gliomas and epilepsy has migrated from studies on human glioma cell lines to animal models in which human glioblastoma cells are xenografted into cortex of immunodeprived mice.62 More recent mouse models have been constructed by genetic deletion of tumor suppressors, such as AKT and the V12 mutated guanosine triphosphatase HRas (HRasV12), permitting exploration of cell-type-specific tumors.63,64

Face validation criteria for the xenografted model are satisfied in that glioma cells proliferate, migrate, and interact with the host brain, and peritumoral cortical tissue generates seizure-like activity. In mice, as in patients, elevated glutamate levels promote glioma cell proliferation, induce excitotoxic neuronal death, and facilitate epileptic synchrony.62,65 Glutamate release at synapses made by neurons on glioblastoma cells in both xenografted mice and human tumor biopsies also drives glioma cell proliferation.66,67

3.3 Models of inherited epilepsies linked to cortical malformations

Focal cortical dysplasias (FCDs) are cortical malformations, sometimes with a defined genetic origin, associated with seizures that typically emerge in infancy.68 Anatomical defects including cortical dyslamination and large, disoriented neurons and glial cells were first identified in postoperative tissue.69 Distinct subclasses of FCD (I, II, III) have been defined on imaging and histological criteria. FCD II has been linked to mutations 70–72 in elements of mammalian target of rapamycin (mTOR) pathways. Cortical tubers, a distinct malformation, are also linked to mutations in proteins of the mTOR complex.73,74 Epileptic syndromes of the cortical dysplasia spectrum are thus associated with aberrant cell types and anatomical malformations that may be linked to defects in mTOR signaling.75

First generations of animal FCD models were based on treatments, including irradiation in utero and cryogenic lesions, which induced dysplasias but only weak epileptic activity. More recent models have been constructed by the expression in mice of human mutations of genes coding for the mTOR molecule70 and adjunct molecules including the DEP domain containing 5 (DEPDC5)71 and the tuberous sclerosis complex molecules 1 and 2 (TSC1, TSC2).73,74 Anatomical and epileptic pathologies are reproduced in mice by the expression of mutated human genes coding for elements of mTOR signaling pathways.

Face validation in mouse models of FCD based on human mutations have been successful in that they largely reproduce abnormal cellular phenotypes, cortical malformations, and epileptic activities.70,74,76 The TSC and DEPDC5 mutations reproduce an uneven spatial distribution of dysplastic cells in the brain, as in patients. This property may have a basis in a genetic double hit resulting from both germline mutations, and somatic mutations, expressed in mosaic fashion by certain cells.76,77

4 HUMAN POSTOPERATIVE EPILEPTIC TISSUE

Human brain tissue has been studied (Figure 2) after operations on patients with acquired focal epilepsies of the mesial temporal lobe (MTLE)78 or cortex,79 with cortical tumors,80 and with cortical malformations.81 Postoperative tissue has been used extensively in anatomical studies of cell types, and molecular expression. Living human epileptic tissue opens the possibility to measure basal activity of an epileptic tissue ex vivo and also to define the physiological and molecular responses to controlled stimuli.

The timing and quantity of tissue available depend on surgical criteria. Operative techniques are key to obtaining viable nervous tissue and good data. Work on acute slices from MTLE patients is usually done when antiseizure drugs become ineffective. In contrast, surgery on glioma patients is performed at an earlier stage in the progress of the disease. Tissue is not available for syndromes that are not treated surgically, such as absence seizures,82 or the inherited Dravet syndrome.83

Slices prepared from tissue (Figure 2A) obtained from patients with MTLE,84 peritumoral regions of cortical slices (Figure 2B) from patients with gliomas,59,80 and tissue from patients (Figure 2C) with FCDs85,86 or tuberous sclerosis87 often generate population activities spontaneously. The duration of these events is 50–100 ms, similar to human sharp waves83 or interictal events.89 Ictal-like discharges with a duration of several seconds are induced by convulsants such as 4AP or ionic changes that increase excitability.90 Distinct patterns of seizure onset in records from slices resemble those seen in EEG traces obtained in situ with intracranial electrodes.91 The generation of realistic interictal and ictal-like activities and transitions between them in slices
points to the role of local-circuit interactions in all three phenomena.

Experiments on human tissue are constrained compared to those on animal models. Recording techniques have improved with attention to cutting slices, oxygenation, and medium composition. The absence of healthy control tissue is a serious problem. Control tissue would help validate the pathological nature of activities generated by tissue from an epileptic patient. Nonepileptic neuronal synchronies are generated by epileptic cortex and also by cortex from tumor patients without seizures. They may represent an early or sub-threshold pattern, missed in vivo. However, synchronous epileptiform events are recorded at similar sites in successive slices, suggesting they depend on neuronal or network defects that extend beyond a single slice. Further, ictal-like events are only recordable in tissues from epileptic patients and are mostly elicited in tissues with interictal synchronies, stressing the pathological nature of these tissues. Substitutes for control tissue include tissue excised surgically en route to a resection target or nonepileptic peritumoral cortex. Another validating procedure is to compare putative epileptic activities generated in slices with EEG traces from the same patient.

One shortcoming of work on acute slices of human tissue is that molecular tools based on viral vector-mediated protein expression cannot be used. The lifetime of living human slices is limited to 1 or 2 days. However, improved techniques for organotypic culture of human tissue permit the use of viral vector-mediated protein expression. These cultures maintain all cell types in a correct spatial context. The anatomical and physiological properties of cultured, adult human tissue are conserved over several weeks. This preparation has enabled optogenetic control of firing in identified neurons and imaging of Ca transients in specific cell types.
5 | COMPARISONS BETWEEN ANIMAL MODELS AND HUMAN TISSUE

5.1 | Anatomical data

Anatomical findings on postoperative human tissue have both confirmed and stimulated work on animal models. Thus, reactive axonal growth and aberrant synaptogenesis was first established in the mouse using the Timm stain to show that mossy fiber terminals terminated on dentate granule cells after kindling. This was confirmed when the same technique revealed “recurrent” synapses made with dentate cells of postoperative tissue from MTLE patients. Similarly, a subset of GABAergic inhibitory cells, containing the peptide somatostatin (SST), were found to die after dentate kindling in rats. Confirmation came shortly later, when SST immunopositive GABAergic cells were shown to be absent in the dentate region of human postoperative tissue. In some cases, findings originally derived from human tissue were confirmed in animal models. For instance, cortical dyslamination with large disoriented neurons, balloon cells, and very large astrocyte-like cells was first detected in tissue from epileptic patients with focal dysplasia. Similar anatomical anomalies were later induced in an irradiation-based rat model of FCD. Such cross-confirmations enhance the strength of findings from human and animal studies.

5.2 | Antiseizure molecules

One strong element of the predictive validity of animal models is whether potential antiseizure molecules translate into approved medicaments. Preclinical trials of novel molecules are based on tests on animal models, derived tissues, and cells. Clinical trials comprise several phases of tests on humans. They include tests in healthy subjects as well as comparisons of a potential new molecule to placebo and current-best-care drugs in patients with the disorder of interest. They are regulated by the European Medicines Agency in Europe and by the US Food and Drug Administration (FDA).

The FDA approved 273 new therapeutic molecules between 2015 and 2020 (Center for Drug Evaluation and Research: https://www.fda.gov/about-fda/fda-organization/center-drug-evaluation-and-research-cder). Approximately 13% of new molecules were linked to brain disorders. The delay from initial preclinical trials until approval may be as long as 8–10 years. The FDA has approved 16 antiseizure molecules to treat partial onset seizures in adults since 1989. Approved treatments comprise zonisamide, 1989; vigabatrin, 1989; lamotrigine, 1990; oxcarbazepine, 1990; gabapentin, 1993; felbamate, 1993; topiramate, 1995; tiagabine, 1996; levetiracetam, 1999; pregabalin, 2004; lacosamide, 2008; eslicarbazepine, 2009; retigabine, 2011; perampanel, 2012; brivaracetam, 2016; and cenobamate, 2019. The rate of approval is slowing: 10 of them were approved from 1989–2005 and only six from 2006–2022. Approval has been restricted or withdrawn for a minority of newly approved drugs, after significant side effects were detected. The efficacy of recently approved molecules in preventing seizures is not markedly improved.

How well do predictions fare for therapeutic molecules derived from models of the epilepsies examined here? Linkage of pathways involving mTOR to cortical dysplasias has provided novel drug targets. An analogue of the mTOR antagonist rapamycin has been approved by American and European agencies as adjunctive therapy for TSC-associated focal seizures. Strategies to counter peritumoral epilepsies have targeted the xc cysteine/glutamate exchanger, which should have antiseizure effects by reducing extracellular glutamate. However, clinical trials of antagonists of the exchanger have not been convincing. Novel targets to suppress seizure initiation could enhance drug development for acquired epilepsies such as MTLE. However, novel molecules have not routinely been tested in chronic animal models of acquired epilepsies. Even if screening and validation may be more complex and time-consuming in these animal models, realistic differential effects of antiseizure drugs are retained, suggesting that models such as KA-treated mice could provide data needed to improve drug efficacy.

5.3 | Pathological mechanisms

A second element of predictive validity is that mechanisms derived from animal models should reproduce those of human tissue. We examine this point by comparing findings from human tissue with data derived from animal models of the same conditions.

5.3.1 | Depolarizing effects of GABA promote epileptic activity in human MTLE tissue

Depolarizing postsynaptic actions of GABA (Figure 2A) have been detected several times in hippocampal tissue from MTLE patients, but relatively neglected in work on animal models. In MTLE tissue, depolarizing inhibitory postsynaptic potentials (IPSPs) in a minority of subicular pyramidal cells act together with glutamatergic excitatory postsynaptic potentials to generate interictal-like...
pyramidal cells, a higher proportion than in MTLE tissue. Depolarizing GABAergic events were confirmed in slice models of acquired hippocampal epilepsies. Depolarizing GABAergic signals may also contribute to seizure initiation. Optogenetic activation of inhibitory cells facilitates ictal events in mice, and intracranial records suggest seizures in MTLE patients are preceded by intense firing in presumed interneurons.

5.3.2 | Depolarizing effects of GABA and epileptic activity in peritumoral cortex and dysplasia

K-Cl cotransporters are dysregulated in pyramidal cells of peritumoral cortex (Figure 2B). KCC2 expression is reduced, whereas NKCC1, a Na-K-Cl, Cl-importing transporter, is upregulated 60% of pyramidal cells, a higher proportion than in MTLE tissue. Depolarizing IPSPs precede interictal-like events in pyramidal cells of peritumoral cortex.

Excitatory GABAergic signaling was confirmed in the xenographed mouse glioma model. GABA reduces Cl− levels in glioma cells and tends to suppress mitosis and migration. Optogenetic stimulation has shown that fast-spiking interneuron firing reduces proliferation, whereas pyramidal cell activity increases glioma growth. Frequent seizures, and presumably high ambient levels of GABA, tend to slow tumor growth.

GABA also exerts depolarizing actions in tissue from patients with FCDs (Figure 2C). NKCC1 expression is enhanced in dysplastic cells, and KCC2 is internalized rather than expressed at membrane sites. Interictal-like synchrony in FCD tissue is driven by rhythmic GABAergic events and suppressed by the NKCC1 antagonist bumetanide.

5.3.3 | Pannexin 1 is proepileptic in peritumoral cortex

Pannexin 1 channels are expressed by neurons, astrocytes, and microglia and mediate exchange of ions and small molecules, including ATP, glutamate, and GABA. They open in response to depolarization, hypoxia, ATP, and glutamate.

Pannexin 1 channels bind to the purinergic receptor 7 (P2X7) to form a multimolecular pore for ATP and cytokine release. ATP release via the pannexin/P2X7 pore should be proepileptic. However, changes in seizure susceptibility in animals in which elements of the pore are genetically modified are contradictory. In P2X7 knockout (KO) animals, seizure susceptibility was enhanced, whereas in pannexin 1 channel KO animals, ATP release was attenuated and seizure severity was reduced.

Pannexin 1 expression is enhanced in neurons, dysmorphic cells, and glial cells in tissue from FCD patients. In living FCD tissue, the pannexin 1 channel antagonists, probenecid and mefloquine, suppressed ATP release and blocked seizurere-like activity. Pharmacological suppression and genetic invalidation of pannexin 1 have since been shown to suppress seizurere-like activity in animal models.

5.3.4 | CXCL8 is the major inflammatory mediator liberated after a human seizure

In response to a seizure, activated glial cells release pro-inflammatory cytokines. The most strongly released cytokine in rodents is interleukin 1-beta. In contrast, the maximally released cytokine after a human seizure in situ is the C-X-C motif chemokine ligand 8 (CXCL8). Furthermore, seizurere-like activity induced in naïve slices of human hippocampus increases CXCL8 protein levels. CXCL8 is strongly released after febrile seizures in children, and after traumatic brain injury and ischemic stroke in adults.

CXCL8 is not released in rodents. Whereas it is present in genomes from the zebrafish to the human, the absence of the gene encoding for CXCL8 from the rodent genome seems to reflect a selective deletion event. CXCL8, a neutrophil activator, should facilitate neutrophil extravasation and stimulate angiogenesis in humans. In rodents, other cytokines might activate receptors sensitive toCXCL8. However, CXCL8 seems likely to enhance immune responses to a human seizure. Neutrophil mobilization is enhanced when CXCL8 is restored in mice by forced expression of human CXCL8 or by treatment with recombinant CXCL8.

5.3.5 | Purinergic effects on microglial processes diverge in humans and rodents

Mobile microglial processes extend toward sites of brain damage in rodents. This response is initiated by purinergic signals from the damage site, which activate the purinergic receptor 12 of the P2Y family (P2Y12). In an inflammatory state, P2Y12 receptor expression is suppressed, and the adenosine receptor subtype 2A is upregulated.
In response to damage, microglial processes of an inflammatory brain retract due to activation of A2A receptors.\textsuperscript{149} Human MTLE and cortical glioma tissue is also in an inflammatory state, but microglia express P2Y12 receptors.\textsuperscript{135,136} Purines, at low levels, induce P2Y12 receptor-mediated process extension.\textsuperscript{148} Receptor expression and motility in these tissues therefore differs from that of rodent microglia after exposure to lipopolysaccharide.\textsuperscript{147} At higher levels of purines, human microglial processes do retract, a response mediated by purinergic receptors 1 and 13 of the P2Y family (P2Y1 and P2Y13) rather than adenosine A2A receptors.\textsuperscript{150}

Some inflammatory states may differ in mouse and human, and differences may also exist between human pathologies. P2Y12 protein expression is unchanged in MTLE tissue\textsuperscript{151} and immunostaining persists in MTLE and cortical glioma tissue. In contrast, P2Y12 receptor immunostaining is reduced in microglia around amyloid-beta plaques of Alzheimer patients\textsuperscript{152} and active lesions in multiple sclerosis.\textsuperscript{153} At 1–2 days after kainate-induced epilepsy, mouse microglia are activated and purine-induced process extension is enhanced.\textsuperscript{154} This state may be similar to an alternative activation phenotype.\textsuperscript{155} Transcriptomic analysis of human MTLE tissue, which has suffered strong neuronal loss over many years, suggests that protective elements of microglial phenotype are enhanced.\textsuperscript{135}

6 | CONCLUSIONS

We have suggested that animal models of epileptic disorders could be judged on their predictions for new medications and mechanisms. Relatively few new molecules have been approved recently to treat partial seizures in adults. They typically do not possess enhanced additional efficacy or disease-modifying effect, so they may best be classified as antiseizure medications. Possibly, new targets such as the K-Cl cotransporters could be examined.\textsuperscript{117,156,157} Pannexin 1 blockers may constitute a new therapeutic target. The identification of targets of mTOR pathways for genetic dysplasias is promising. Everolimus, an analogue of rapamycin, the mTOR antagonist, has recently been approved by the FDA. In principle, human tissue, cells, or cultures might be a useful adjunct in testing procedures for novel molecules even though the logistics of availability, standardization, and how best to provoke a seizure would need to be resolved.

Comparison of mechanisms derived from work on human tissue and on animal models uncovered points of interest. The emergence of a role for Cl- cotransporters dysregulation in epileptic activities in MTLE, FCD and peritumoral tissue as well as for pannexin 1 recall the cross-fertilization of anatomical data from postoperative tissue and work on models. In contrast, the release of CXCL8 after a seizure seems to be a clear-cut difference between human and model. Further work is needed to resolve whether the difference in purinergic effects on microglial motility is clear-cut or derives instead from the multiplicity of inflammatory phenotypes in both humans and animals. Overall, the comparison supports a rigorous cross-verification of findings, to avoid the assumption that mechanisms should be identical.

The limits of work possible with human tissue are progressively being reduced. Organotypic cultures can be maintained over several weeks,\textsuperscript{96,101,102,103,104} permitting the use of molecular tools impossible in acute slices of living tissue. Induced pluripotent stem cells may be cultivated as organoids based on human cells from patients with specific conditions.\textsuperscript{107,158} Such novel approaches should facilitate understanding of common mechanisms as well as the emergence and validation of more effective antiseizure molecules. Even so, it may remain true that “the best model of a cat is another, or preferably the same cat.”\textsuperscript{159}

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CONFLICT OF INTEREST STATEMENT

None of the authors has a conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID

Gilles Huberfeld  
https://orcid.org/0000-0001-8120-0300

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