Limitations of Animal Epilepsy Research Models: Can Epileptic Human Tissue Provide Translational Benefit?

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Abstract

Advancement of understanding the etiology and treatment of epilepsy has largely depended on the use of acute and chronic animal models. An alternative approach, which is being increasingly used by a select number of laboratories worldwide, is to perform functional mechanistic studies in brain slices of living human tissue resected during surgery for drug resistant epilepsies. Pharmacoresistant epilepsy is a major clinical problem with a significant proportion of patients not receiving any symptomatic benefit from available anti-epileptic drugs. Animal models of epilepsy have dominated the landscape with regard to research and development, however they have failed to deliver new agents that would provide seizure control in patients with drug refractory epilepsy. Moreover, these models have considerable issues with respect to validity and animal welfare considerations. A compelling alternative is the use of live human epileptic tissue, which recapitulates a number of key features of refractory epilepsy. The use of live epileptic human tissue offers unprecedented opportunities to understand the mechanisms associated with difficult to treat epilepsy whilst also permitting studies of efficacy of novel agents that are being developed to alleviate epilepsy in drug resistant patients.

1 Introduction

Up to 1% of the population suffer recurring seizures and are diagnosed with epilepsy, equating to around 600,000 people in the UK and 50 million worldwide1. A significant proportion of these people (30-40%) are refractory to drug treatment, leaving surgical resection of the identified focus as the most viable alternative (Mohanraj and Brodie, 2006). A better understanding of the disease etiology and improved treatments are highly desirable. Basic or experimental epilepsy research has long made use of animal models, but the usefulness of these is increasingly being questioned (Sloviter, 2005; Sloviter and Bumanglag, 2013). Historically, cats, dogs and non-human primates were more commonly used to study epilepsy, however, since the 1980’s, rodent models have been the dominant species in epilepsy research (Grone and Baraban, 2015). The most recent UK Home Office statistics (UKHO, 2019) show the second highest proportion of animal procedures performed in basic research is for studies of the nervous system (21% of all procedures), with procedures in mice (60%), fish (17%) and rats (9%) comprising the vast majority of these. Unfortunately, there are no records which specify the number of animals used solely for epilepsy research.

An animal model of epilepsy, using electrically induced convulsions in cats, heralded the discovery of the first modern antiepileptic drug (AED), phenytoin, that was subsequently studied in a large cohort of patients (Putnam and Merritt, 1937). Later, other models of epilepsy were developed and proved useful in the search for safer and more efficacious AEDs. This approach was successful in that it produced a second generation of better tolerated and clinically effective AEDs for patients (LaRoche, 2007) (e.g., lamotrigine, levetiracetam, topiramate, lacosamide, pregabalin and others). However, despite the impressive armory of AEDs (ca. 20 medications) that clinicians can avail of for symptomatic treatment, approximately 30% of all epilepsy

1 WHO epilepsy factsheet (2019). https://www.who.int/news-room/fact-sheets/detail/epilepsy (accessed June 2020)
patients remain resistant to treatment by AEDs (Mohanraj and Brodie, 2006).

The most common form of focal intractable epilepsy is mesial temporal lobe epilepsy (MTLE), and it is estimated that ~70% of patients with MTLE are refractory to available AEDs (Engel, 2001; Schmidt and Löscher, 2005). Neuropathological studies have demonstrated that in patients with refractory MTLE, mesial temporal sclerosis (hippocampal atrophy) is the common pathological substrate of the condition (Engel, 1996). Using histopathological approaches in human surgical samples, this atrophy is characterized by neuronal cell loss in the cornu ammonis (CA) 1 and 4 subfields of the hippocampus, dentate gyrus granule cell death, astrogliosis, and extensive reorganization of axons (Blümcke et al., 2013). In the majority of cases, the etiology of MTLE is idiopathic, however it is believed that there is an initial causative injury that can include trauma, febrile seizures, stroke, status epilepticus (SE) or a brain infection (Mathern et al., 1995; Engel, 2001). It is thought that these injuries trigger a neuro-pathological chain reaction that sets off a process of epileptogenesis in the hippocampus and associated structures within the temporal lobe. Following a latent period, which can last for months or years, the patient then presents with epilepsy, which proves to be pharmacoresistant in a significant proportion of cases.

Given the impact of the condition and the impetus to deliver improved symptomatic treatments and possible cures for epilepsy, many researchers have turned to animal models, predominantly using mice and rats, to attempt to understand more about the pathophysiology that gives rise to temporal lobe epilepsy (TLE). Broadly speaking, three forms of animal models are capable of recapitulating some of the electroencephalographic, pathological and behavioral aspects of human MTLE. These involve either the systemic administration (intraperitoneal injection; kainic acid (Ben-Ari et al., 1980), pilocarpine (Turski et al., 1983; Curia et al., 2008)) or topical application (intracerebral injection; kainic acid (Ben-Ari et al., 1979; French et al., 1982), pilocarpine (Millan et al., 1993), tetanus toxin (Mellanby et al., 1977)) of chemoconvulsant agents or the repeated electrical stimulation (kindling) of limbic brain structures (Löschter, 1997, 2011). Additionally, various genetically engineered animal models are available for genetic epilepsies, which are also frequently pharmacoresistant. The pressing challenge for the experimental epilepsy research community is to translate the findings of these preclinical studies into the clinical arena. The major questions that presently dominate this area are i) the elucidation of the mechanisms that explain pharmacoresistance and ii) the discovery of novel compounds that could bring about seizure control in AED refractory patients.

There is strong evidence to suggest that patients with refractory epilepsy (e.g., MTLE) benefit from surgical intervention earlier in the course of the condition rather than later in order to improve the chance of seizure freedom (Wiebe et al., 2001; Engel et al., 2003; de Tisi et al., 2011; Engel, 2012). Considering this, the increased numbers of patients undergoing resective surgery present a unique opportunity for in vitro experimental studies of human epileptic tissue. Whilst there is little knowledge to be gained from this tissue with respect to epileptogenesis, given the end state nature of the tissue, there is much to be gained from this tissue in the context of understanding the pathology that underlies pharmacoresistance. In this review, we will outline the problems associated with examining drug resistant epilepsy using animal models and the advantages of using epileptic human tissue for the purposes of electrophysiological studies to address this clinical problem.

2 Difficulties in recapitulating drug resistant epilepsy in animal models

A key issue is the clinical relevance of animal models for epilepsy research and, in assessing this, the usefulness of a model can be evaluated by using three criteria: construct validity, face validity and predictive validity (van der Staay, 2006). In an ideal world, the perfect animal model would meet all criteria, i.e., demonstrate a similar etiology to that observed in the human condition; demonstrate a similar physiological, genetic and behavioral phenotype; and exhibit a similar response (or lack of) to AED therapies. We will now discuss each of these criteria individually, examining the evidence that supports or refutes the validity in a number of animal models of epilepsy, and summarize our conclusions in Table 1.

With construct validity in mind, inherited mutations in ion channel or synaptic receptor genes contribute significantly to the monogenic causes of idiopathic epilepsy. Using clinical and molecular genetic analysis collected from patients, the construct validity of the molecular etiology can then be assessed by developing an appropriate animal model using genetic engineering techniques. Subsequent experimental epilepsy studies can then be conducted on the animal model in order to gain a better understanding of the mechanisms underlying epilepsy.

Several examples of using this approach currently exist. It is now well established that in Dravet syndrome, the cause in the majority of human cases is a de novo mutation of the SCN1A gene producing a loss of function of the type 1 voltage-gated sodium channel (Na1.1) (Claes et al., 2001, 2003). Using this clinical knowledge, multiple mouse models of Dravet syndrome have been developed (Yu et al., 2006; Ogawa et al., 2007; Miller et al., 2014), and it has been demonstrated that Scn1a<sup>-/-</sup> mice exhibit both spontaneous and hyperthermia-induced seizures (Oakley et al., 2009).

In humans, a dominant-negative missense mutation in a potassium channel gene (KCNA1) produces partial temporal lobe seizures and generalized tonic-clonic seizures (Zuberi et al., 1999). The KCNA1 gene codes for the K<sub>v</sub>1.1 voltage-gated potassium channel. K<sub>v</sub>1.1 is critical for regulating numerous features of neuronal function, including action potential propagation and shape, repetitive firing properties and neurotransmitter release (Tanouye et al., 1981; Zhang et al., 1999; Dodson and Forsythe, 2004). Whilst 50% of the mice generated with a null knockout of the KCna1 gene died suddenly at 3-5 weeks old, they did exhibit what appeared to be generalized seizures before death. The mice that survived beyond this time point continued to display sporadic spontaneous seizures, as measured behaviorally and with EEG recordings (Smart et al., 1998).
Mutations in specific subunits of the NMDA receptor are also emerging from the clinical literature. Specifically, de novo mutations in GRIN2B and GRIN2A, which encode the GluN2B and 2A subunits of the NMDA receptor, are reported in individuals with epilepsy and intellectual disability (Endele et al., 2010). Subsequent electrophysiological studies in Xenopus laevis oocytes have demonstrated that a missense de novo mutation in the receptor pore region (GluN2A(N615K)) is capable of altering the current density of the receptor and the receptor’s sensitivity to exogenous and endogenous modulators (Marwick et al., 2015). It has subsequently been demonstrated that Grin2a knockout mice exhibit spontaneous epileptiform discharges (Salmi et al., 2018).

With advances in molecular and genetic techniques, particularly homologous recombination, a variety of useful insights into the role of mutations of single genes in epilepsy have been revealed. The ability to integrate the specific genetic abnormality derived from human patients (e.g., Ogiwara et al., 2007) has to a certain degree resulted in significant face validity. The models reiterate a critical phenotypic feature (spontaneous seizures) of the human condition. Whilst such Mendelian epilepsies only constitute a small number of all the epilepsies, their clinical burden is significant in that they are frequently difficult to treat with AEDs.

As outlined above, spontaneous seizures have been reported in numerous studies, however a major shortcoming of the work to date with genetically modified mouse models has been a lack of assessment of the predictive validity of these models. It would be worthwhile to test if these models recapitulate non-responsiveness to commonly used AEDs. Undertaking this endeavor would require significant resources, as almost 1000 genes have been associated with epileptic phenotypes (Wang, J. et al., 2017). However, if a large cohort of genetically modified mice exhibiting epilepsy could be phenotypically screened (using EEG and behavioral measurements) to identify individual pharmacoresistant models, this would help to select out mice that demonstrate face validity.

With an increasing number of identified human epileptic mutations and genes leading to an escalation of the number of genetically modified mice, welfare issues are worth considering. Adverse welfare effects associated with spontaneous seizures include weight loss; increased stress and anxiety; hyper-reactivity and aggression. These should be anticipated, and good refinement protocols and control measures used to reduce possible suffering (Lidster et al., 2016). Whilst easy to produce and inexpensive, it is important that genetic modification reproduces the pathophysiological state observed in the human condition accurately. The gene targeting approaches outlined in this section are optimal in that they reliably reproduce the genetics, pathology and phenotype of the human epileptic condition.

In many cases of epilepsy, Mendelian patterns of inheritance play no role in the pathology. These non-Mendelian cases usually arise with sporadic frequency due to traumatic brain injury, brain tumors, developmental abnormalities and vascular insults. Whilst in the case of genetic disorders the development of an animal model is derived from a known molecular etiology, in contrast, non-Mendelian models must demonstrate face validity (i.e., recapitulation of distinct clinical feature(s)). From a clinical perspective, MTLE has received a high degree of characterization, meaning that a significant level of design and appraisal of animal models of this condition can be undertaken.

Whilst MTLE can be heterogenous in terms of etiology, a key feature is conserved across the majority of patients, i.e., the unilateral pattern of hippocampal neuronal loss and gliosis termed hippocampal sclerosis (Blümcke, 2009). In many patients with MTLE, the sclerotic hippocampus can be visualized on magnetic resonance imaging (MRI) or by histopathology using resected tissue obtained from surgery. Localized hippocampal damage (edema, increased T-2 weighted intensity) can be observed days following focal uncontrollable febrile seizures, with subsequent hippocampal atrophy occurring in the months after the initial seizures (VanLandingham et al., 1998). Febrile and afebrile SE in children and adults, respectively, are now thought to be a major factor in the development of epilepsy following a seizure-free epoch of indefinite duration (Annegers et al., 1987; Tsai et al., 2009). Indeed, an analysis of a cohort of 67 patients undergoing elective neurosurgery for refractory MTLE demonstrated that the majority had SE preceding the onset of their epilepsy (French et al., 1993).

From a pre-clinical perspective, a variety of animal models of SE provoked TLE have been developed. Broadly, these can include the systemic or topical administration of chemoconvulsants or direct electrical stimulation of the brain. Systemic convulsants are useful as they can trigger epileptogenesis, with face validity similar to human MTLE. Topical chemoconvulsants may be used to trigger acute seizures or to trigger epileptogenesis in some cases (see below for a detailed discussion of the intra-amygdala kainic acid model). Electrical kindling can also trigger epileptogenesis through non-chemical targeting of specific brain pathways.

However, only one model would appear to capture several features of the clinical syndrome and therefore go some way in terms of achieving the criteria of face validity. The infusion of the glutamate receptor agonist kainic acid (KA) into the basolateral nucleus of the amygdala (BLA) can produce SE that is subsequently followed by mTLE (Ben-Ari and Lagowska, 1978; Ben-Ari et al., 1979; Lévesque and Avoli, 2013). This model (KA-BLA) produces this epileptic phenotype in both young and adult rodents and usually involves a rapid onset of a period of SE, which is terminated by the administration of a benzodiazepine (diazepam or lorazepam) to decrease the risk of mortality (Sharma et al., 2008, Lévesque and Avoli, 2013). Following a latent period of epileptogenesis lasting several days after the KA insult and SE event, spontaneous recurrent seizures occur and have been documented to persist. The only major difference between the implementation of the model in juvenile and adult rodents is that in young rats SE is permitted to run its course and naturally terminate in both cases, following a significant period of time (weeks to months), both behavioral and electrographic seizure activity is manifest, proving the presence of MTLE. Moreover, unilateral hippocampal sclerosis that coincides with the development of epilepsy has been confirmed using imaging and histopathological techniques.

Whilst these experimental observations support the face validity of this model, some elements are at odds with the clinical syndrome. Firstly, the length of the latent period is vastly differ-
hilus. Finally, it is worth mentioning that the construct validity of this model is flawed. Whilst the model does recapitulate the initiating pathology, that is SE, this is in response to a convulsant agent (KA) rather than febrile illness. It is interesting to note that the C57BL/6J strain of mice and young rat pups (postnatal day 10-11) can exhibit febrile seizures after hyperthermia induced by exposure to warm air (van Gassen et al., 2008). Prolonged febrile seizures induced by this model can go on to produce spontaneous

Tab: 1: Summary of benefits and limitations of resected human tissue from patients with drug-resistant temporal lobe epilepsy versus animal models, IPS cells and computational models

| Human tissue                          | Animal models                      | IPS cells                                      | Computational models |
|---------------------------------------|------------------------------------|-----------------------------------------------|----------------------|
| **Throughput**                        | Low as subject to clinical procedures | High. Animals are typically available when needed. | Medium. Cells/organoids may need a long time to form mature neurons and networks. | Very high |
| **Quality of experimental preparation**| Variable. Clinical outcome must take precedence over research outcome. Tissue can be damaged during resection. Sclerotic tissue is largely unusable. | High. Easily controlled by the researcher. | Preparations may be technically challenging, but are well controlled by an experienced researcher. | Easily controlled by the researcher. |
| **Construct validity**                | High. The experimental preparation is the exact construct for which treatment is required, though may be damaged during resection or removed from wider network connections. | Can be high for some genetic epilepsies, where the exact human mutation can be modeled in mice. Lower for MTLE models, where the causative insult in humans is often unknown and unlikely to be reflected in mouse models. | High at the cellular level; variable at the network level. The cells are derived from human material, but do not necessarily form realistic or mature neuronal networks. | Low as epilepsy is modelled using computers and not biological substrates. |
| **Face validity**                     | Resected slices can generate spontaneous interictal activity, though seizure-like activity usually must be evoked by chemoconvulsants and may not reflect the pathological network activity that caused seizures in the patient. | Models can capture epileptogenesis and show spontaneous seizures that appear to reflect those seen in humans. Other behavioral characteristics or co-morbidities may not be represented. | Can capture cellular features of genetic epilepsies. Organoids can generate spontaneous network activity. No evidence of spontaneous seizures. | Can capture micro- or macroscopic biophysical features, but usually not both at the same time. |
| **Predictive validity**               | Good. Tissue is resistant to AEDs, a property not seen in brain tissue resected from non-epileptic cases. | Limited evidence. In some models, 30-40% of animals show pharmacoresistance, in line with human MTLE. | Difficult to assess as current models do not exhibit spontaneous seizures. Some evidence at the cellular level in Dravet syndrome | Further work is required to assess this. |
| **Ethical considerations**            | Informed patient consent and specific ethical approval is required. | Project and personal licenses are required for animal procedures. | Human-derived cells may be subject to existing ethical agreements. | Less applicable as not using biological tissue. |
electroclinical seizures in a third of the adult rats (Dubé et al., 2006). Further work is required to demonstrate the overall validity of this particular model.

The major limitation of this epilepsy model and others concerns predictive validity. Predictive validity can be defined as the effectiveness of research studies or tests to predict the outcome of future interventions. This definition can be used to argue that predictive validity is when the animal model recapitulates treatment responsiveness. However, given that a key clinical problem in MTLE is pharmacoresistance to AEDs, perhaps it is worth reconsidering the concept of predictive validity in this context. High predictive validity is how closely an animal model recapitulates AED responsiveness that is observed in humans. We would posit that we should consider high predictive validity of a preclinical animal model of MTLE as its ability to demonstrate it to be non-responsive to AED therapy. This observation would bring a preclinical animal model in line with the clinical definition of pharmacoresistance. To that end, it is critical that the pharmacological responsiveness, or not, of spontaneous seizures is examined in an animal model of human MTLE. Unfortunately, this has been a poorly studied area of epilepsy research, partly due to difficulties associated with attempting this type of work. The testing of the efficacy of AEDs is laborious, time consuming, expensive and complicated by differences in pharmacokinetics between rodents and humans. Prolonged video telemetry recordings of rodents are also required to compare seizure incidence during periods of no drug versus seizure frequency during epochs when animals receive AED treatment.

Using the pilocarpine model of MTLE, it was found that rats demonstrated a significant inter-individual variation in responses to levetiracetam administered via an osmotic pump (Glisen et al., 2002). Up to 40% of rats responded to the drug with virtually complete control of spontaneous seizures, 40% of the rats tested were non-responsive to the AED, whilst the remainder showed so much variation in seizure control pre- and post-drug that they were excluded. In a separate study, rodents exhibiting spontaneous recurrent seizures induced by BLA electrical stimulation (kindling) could be divided into responders and non-responders to phenobarbital (Brandt et al., 2004). In other studies, this finding was replicated with 30-40% of rats non-responsive to phenobarbital. A significant proportion of these non-responders were also resistant to subsequent treatment with phenytoin (Löschner, 2002).

The International League Against Epilepsy (ILAE) defines pharmacoresistant epilepsy as failure of two tolerated (maximum doses), appropriately chosen and used AEDs (whether as mono-therapies or in combination) to achieve protracted seizure freedom (Kwan et al., 2009). In light of this description, it would appear that these limited studies demonstrate predictive validity in the form of AED non-responsiveness. However, it should be noted that both the pilocarpine model and the BLA electrical stimulation model have additional limitations with respect to validity. From an electrophysiological perspective, there is a much smaller degree of variation in seizure onset sites in human patients as compared to rats that have been exposed to pilocarpine (Toyoda et al., 2013). Moreover, in humans with MTLE, the lesion and onset are usually lateralized, whereas in the pilocarpine model the lesion and onset are found equally in left and right hemispheres. It should be noted that whilst the lesion created by pilocarpine is bilateral, the neuronal degeneration in CA3/CA1 (Covolan and Mello, 2000) and mossy fiber sprouting (Shibley and Smith, 2002) are reminiscent of the neuropathological findings reported in human MTLE. In contrast, it is generally accepted that the various neuropathological changes observed in human MTLE are virtually absent in the BLA kindled model (Mathern et al., 1997; Brandt et al., 2003).

Due to the nature of inducing experimental models of MTLE, i.e., induction of SE, a process which in isolation can exert significant mortality, there are a number of animal welfare issues that arise from this type of work (Lidster et al., 2016). Using the KA-BLA model as an example, the generation of animals with epilepsy using this approach is associated with several adverse effects and co-morbidities, including potential issues with stereotoxic injections into the brain (death due to anesthesia, post-surgical infection, post-surgical pain, failure of sutures, dehydration). If animals are to undergo EEG video-telemetry, there is risk associated with brain inflammation and infection due to foreign bodies (electrodes) causing alterations in rodent behavior following recovery from surgery. The development of spontaneous recurrent seizures can, as outlined for genetically altered mice above, cause serious adverse effects. Finally, the long-term administration of pharmaceutical agents (i.e., AEDs), particularly at maximally tolerated doses, has the potential to cause deleterious and unexpected effects in animals.

3 Alternative methods of addressing drug resistant epilepsy

Considering the issues highlighted above concerning validity with respect to animal models of drug resistant epilepsy, it is important that we identify alternatives that are more relevant to the human condition. Apart from the obvious animal welfare issues described in previous sections, there are other persuasive reasons to search for different options available to experimental scientists with an interest in epilepsy. The last number of years have seen considerable numbers of animals being used in pre-clinical studies, particularly in studies testing novel compounds in development. Despite this dramatic increase in animal use and budgets associated with pharmaceutical industrial research and development pipelines, the number of compounds that transition to clinical trials or are licensed for therapeutic use, particularly in the central nervous system (CNS) domain, is currently stagnant. Coupled to the lethargic condition of drug discovery pipelines is the number of late stage failures and high profile recalls of potentially successful drugs (Gribkoff and Kaczmarek, 2017). This evidence would suggest that, in general, pharmaceutical research and development urgently requires novel strategies that complement or move away from traditional animal-based biomedical research. This is most pertinent with regard to the field of epilepsy. Notwithstanding the array of experimental approaches and variety of pre-clinical epilepsy animal models developed over many decades, clinicians are still faced with 30% of patients who are...
refractory to commonly used AEDs. With respect to this clinical bottleneck, alternative approaches could include induced pluripotent stem cells (iPSCs) and computational modelling. However, the most plausible alternative to animal models of epilepsy is the use of live human epileptic tissue for in vitro functional studies as a means of predicting drug efficacy and aiding the drug discovery process.

iPSCs can be derived from patient skin biopsies or blood samples and can subsequently be differentiated into neurons, glia, or other cell types of interest. These can be grown in two-dimensional cultures or in three-dimensional scaffolds to generate cerebral organoids with physiologically realistic brain architectures (Benito-Kwiecinski and Lancaster, 2019; Niu and Parent, 2020). The resulting cells contain the same genetic material as the original human tissue sample. This mediates a high degree of construct validity in the context of genetic epilepsies, where seizures are caused by specific gene mutations, and many such iPSC models have been developed (Simkin and Kiskinis, 2018; Niu and Parent, 2020; Sterlini et al., 2020). However, in the context of TLE, both two-dimensional iPSC cultures and brain organoids have limited construct validity since the underlying pathophysiology of acquired epilepsies does not have a purely genetic basis. Regarding face validity, iPSC-based models can capture some important phenotypic aspects of genetic epilepsies. For example, iPSCs derived from Dravet syndrome patients show hypoactivity in interneurons, whilst excitatory neurons appear to be unaffected (Sun et al., 2016), and iPSCs carrying a KCNT1 mutation show increased network excitability and synchrony in two-dimensional culture (Quraishi et al., 2019). Brain organoids cultured for > 8 months exhibit spontaneous neuronal network activity (Quadrato et al., 2017), however current evidence that brain organoids can generate spontaneous epileptiform seizures is limited. This may limit the face validity of protocols for modelling epilepsy in organoids at present. It is challenging to assess the predictive validity of iPSC models in testing anti-seizure therapies since seizures are not well re-capitulated in current versions of these models. However, there is some evidence supporting predictive validity. For example, cannabidiol (CBD) is a promising therapeutic in children with Dravet syndrome. Correspondingly, in iPSC models of Dravet syndrome, CBD reduces excitatory neuron activity whilst boosting inhibitory cell firing (Sun and Dolmetsch, 2018), suggesting that iPSC models can reproduce certain therapeutic actions seen in patients.

Computational models of epilepsy offer alternatives in terms of examining the cellular, synaptic and network properties underlying seizure generation (Lyttton, 2008). Computational models range from those that capture microscopic biophysical features (e.g., changes in particular ionic gradients and dynamics) but do not reproduce macroscopic features to models that faithfully represent network features (e.g., EEG patterns associated with epilepsy) but lack a full consideration of the underlying physiological processes. The utility of computational models of epilepsy in addressing refractory epilepsy is debatable. Recent computational studies have helped to bridge the gap between the microscopic and macroscale regarding seizure generation (Liou et al., 2020). However, further work is required to ensure that such seizure models capture activity that is resistant to parameter changes that could be considered as anti-seizure medication interventions. Moreover, the value of a biologically realistic model is enhanced by the ability to incorporate “real world” and detailed neurophysiological characteristics. Therefore, an iterative process via collaboration between computational and experimental neuroscientists, particularly those focused on understanding the biology of the human epileptic brain, is required to ensure future “in silico” seizure models fully capture the disease condition.

Despite these alternatives to animal models of epilepsy, resective human tissue remains a leading candidate for the reduction and replacement of animal models. The issue of functionality of human tissue is an important one. A large number of human brain tissue samples removed during neurosurgery are ultimately used for diagnostic purposes. Previously, this has meant that limited scientific information has been derived from this resource, as histopathological stains and molecular techniques are used to examine such samples. Whilst this is useful in providing a molecular basis for observed phenotypic variations in the pathological condition, it tells us little regarding the functional changes that correspond to cellular, synaptic and network activity in the human epileptic brain. From a functional perspective, epilepsy remains a disease of the brain that arises due to excessive neuronal activity, and it is through this neuronal activity that AEDs will exert their therapeutic effect, or not. For those reasons and the fact that electrophysiological studies in patients (e.g., EEG) remain the diagnostic “gold standard”, the focus of this section on human tissue will review the use of electrophysiological techniques using epileptic human brain slices in vitro (e.g., extracellular local field potential (LFP) recordings).

The very reason that human brain tissue is surgically removed to treat drug resistant epilepsy provides a unique opportunity for neuroscientists to undertake in vitro research on this valuable resource. Using human brain tissue in this way allows for the replacement, or at least reduction (Flecknell, 2002), of the use of animal models to study pharmacoresistant epilepsy. The fact that this tissue is derived from patients and is likely to capture the causal neuronal mechanisms of epilepsy in humans supports the construct validity of live human epileptic tissue.

Regarding face validity, several phenotypic characteristics can be derived from epileptic human tissue that also support this approach. One limitation of using the human in vitro brain slices approach to study epileptic activity is the lack of correlate with the behavioral and clinical seizure phenotype. However, recent work has demonstrated that a particular type of neuronal oscillation observed in vivo recordings of human epileptic brains is conserved at the level of a human epileptic brain slice (Staba, 2013). This activity is strongly associated with epileptogenic networks in patients with MTLE. Recent work has suggested that pathological high frequency oscillations (HFOs) may represent a unique biomarker that could aid in the localization of brain regions to be resected during epilepsy surgery (Staba, 2013). HFOs can be considered as LFP oscillations whose frequency is greater than 80 Hz, extending up to frequencies of ca. 500 Hz. Clinically, HFOs can be detected using high-sampling rate scalp or intracranial (depth or sub-dural electrodes) EEG approaches. They can be measured experimentally in resected human brain sections using standard
extracellular local field potential recordings (Jones et al., 2016). Moreover, HFOs associated with interictal events are intimately correlated with seizure onset zone in drug resistant epilepsy patients (Jacobs et al., 2010; Wu et al., 2010; Akiyama et al., 2011; Cho et al., 2012; Haegelen et al., 2013; Okanishi et al., 2014). Given this profound association between HFOs and seizure generation, a better understanding of the neuronal behaviors that generate HFOs will provide information that could be used to pharmacologically target HFOs and potentially overcome the issue of drug resistant epilepsy by developing compounds that target the mechanisms critical for this pathological oscillation.

In this respect, several laboratories have exploited the use of epileptic human brain slices to study the mechanistic nature of HFOs. Initially reported by Köhling et al. (1998) in epileptic human neocortical slices, HFOs are tightly correlated with interictal sharp wave events. This study examined the cellular and synaptic features of the sharp wave events, concluding that they are mediated primarily through non-NMDA and GABAergic mediated synaptic activity. More recently, Roopun et al. (2010) recorded spontaneous HFOs (100-500 Hz) in association with interictal sharp waves in human neocortical slices obtained from MTLE patients. Alongside a computational network model, this work demonstrated a weak correlation between chemical synaptic conductance and HFOs. They also observed that antagonism of gap junctions abolished HFOs, whereas the application of a GABA receptor blocker had no effect. In a subsequent study using a similar dataset, HFOs were divided into ripple (<200 Hz) and fast ripple (>200 Hz) components (Simon et al., 2014). Using a multi-electrode array recording approach, it was shown that both forms of activity were predominant in the superficial (II/III) layers of the neocortex. Concurrent extracellular recordings and intracellular measurements of principal cell membrane potential revealed that, whilst excitatory postsynaptic potentials (EPSPs) occur during fast ripple HFOs, there was no significant correlation between synaptic activity and the network HFOs. This finding supports the hypothesis that human fast ripple HFOs are generated by a gap junction coupled plexus of axons (Roopun et al., 2010; Traub et al., 2011, 2014; Cunningham et al., 2012; Simon et al., 2014).

Given that the tissue in the aforementioned studies was obtained from pharmacoresistant cases, this suggests that HFOs are a strong biomarker of disease activity in refractory epilepsy (Staba, 2013). Most AEDs that will have failed in these refractory cases are known to target neuronal ion channels (sodium, potassium) or synaptic receptors (GABA, glutamate). If axonal gap junctions, which are not targeted by conventional AEDs, are important for human epileptogenesis, then it would be reasonable to suggest that the development of compounds that selectively antagonize the axonal gap junction could bring about therapeutic benefit. Experimental studies have demonstrated that a gap junction blocker, carbenoxolone (CBX), suppresses HFOs in epileptic human tissue (Roopun et al., 2010). However, whilst approved for use in humans, it is unclear if CBX can pass the blood brain barrier (BBB). However, it is encouraging that two orally bioavailable gap junction blockers, tonabersat and carabersat, have been shown to have anticonvulsant action in preclinical animal models (Upton et al., 1997). It would be intriguing to test the anti-epileptic potential of these compounds in epileptic human tissue and their impact on HFOs.

The predictive validity of any model is an important yardstick for translational studies. Predictive validity can be considered as the ability to predict that the effect (e.g., of a novel AED) in the assay will be reflected in the patient condition. Additionally, predictive validity can be thought of as the equivalence of disease mechanisms (e.g., ion channel mutation) and pathophysiological features (e.g., imbalance between inhibitory and excitatory neurotransmission) that are similar across the model and the human condition. Given the source, scientific studies in this tissue are advantageous from the point of view of validating pharmacoresistance and probing potential mechanisms underlying this finding. As suggested above, high predictive validity of epileptic tissue from AED refractory patients should be their non-responsiveness to therapy.

There are already precedents for examining this question in the literature. Jandová et al. (2006) have demonstrated that in epileptic human hippocampal brain slices obtained from drug-resistant MTLE patients, induced epileptiform activity recorded as extracellular field was resistant to a commonly used AED, carbamazepine (CBZ). Moreover, they also showed that in tissue from patients not resistant to AEDs (tumor patients), CBZ could suppress induced ictal spiking. In an earlier study, using whole-cell patch clamp recordings conducted in hippocampal neurons from CBZ resistant patients, it was shown that the use dependent block of sodium channels was lost in these patients (Benardo, 2003). Alongside these single cell studies, extracellular recordings of epileptic activity were also insensitive to CBZ. In contrast, in a small number of samples from patients clinically responsive to CBZ, use-dependent block of sodium channels and suppression of epileptic events was observed (Remy et al., 2003).

An alternative hypothesis regarding pharmacoresistance centers around the role of multitudrug transporter proteins (MDTs). MDTs include multidrug resistance-associated proteins 1-5 (MRP1-5) and P-glycoprotein (Pgp), and anatomical studies in resected epileptic human brain tissue have reported an upregulation of these proteins in brain tissue and BBB from drug refractory epilepsy patients (Aronica et al., 2004, 2012). The transporter hypothesis outlines a scenario whereby the accumulation of tissue AED concentrations is obstructed by the efflux of drug out of the neuropil and BBB, facilitated by MDTs. In order to test whether Pgp and MRPs contribute to AED resistance in epileptic human hippocampal and cortical tissue, Sandow et al. (2015) examined a number of AEDs and unspecific blockers of MDTs. They observed that in the presence of CBZ, phenytoin (PHT) and valproate (VAL) induced (reduced GABA_A function and increased extracellular potassium concentration) epileptiform activity was unaltered. It was also reported that non-specific inhibitors of Pgp and MBP (verapamil and probenecid) were also found to not alter induced epileptic activity. Moreover, co-administration of AEDs and drug transport inhibitors failed to suppress activity in the majority of samples. Taken together, these findings suggest that the presence of MBP and Pgp in the neuropil does not underlie the refractory nature of resected tissue.
One final hypothesis should be considered that may be addressed using epileptic human brain tissue, i.e., the network hypothesis, which proposes that structural brain alterations and/or network changes (e.g., hippocampal sclerosis) are involved in resistance to AEDs (Fang et al., 2011). Profound structural and functional abnormalities in neuronal networks are found in refractory epilepsy. Overall, these changes can be considered alterations in brain plasticity and include axonal sprouting (Mello et al., 1993), synaptic reorganization (Sutula et al., 1988), aberrant neurogenesis (Goldberg and Coulter, 2013) and gliosis (Devinsky et al., 2013). In particular, studies on post-mortem and surgical resection samples from patients with intractable MTLE have demonstrated that astrogliosis is a significant feature of the epileptic brain (Wang, L. et al., 2009). Given that astrogliosis plays a critical role in the formation of scar tissue, which itself will hinder the access of drugs to a lesion, it is possible that astrogliosis may contribute to pharmacoresistance in epilepsy. Another feature that is well established in epileptic human tissue is synaptic reorganization. Mossy-fiber sprouting is a consistent finding, and this mis-wiring of neurons within the hippocampus brings about network hyperexcitability through the enhancement of recurrent excitation (Maglóczky, 2010). It is plausible that this augmentation of excitation within neuronal networks will facilitate pathological synchronization between neurons.

There are, of course, limitations to the use of live human epileptic tissue. In many cases, seizure activity is induced by manipulation with a proconvulsant medium. It is rare to observe spontaneousictal events, and it can be difficult to evoke such events. It is not known if the resistance of induced epileptic activity reflects the refractory nature of spontaneous pathological events in human slices or, indeed, patients for that matter. Gaining access to human tissue can be time-consuming, logistically challenging and hampered by low throughput (Jones et al., 2016). Moreover, tissue specimens can be of a heterogeneous nature, meaning that it can be difficult to get consistency in terms of the anatomical source of the tissue and the phenotype of the patient. However, the amount of epileptic human tissue is substantial, and repeated measures can be achieved from within the same subject. Thus, in rare cases of drug-resistant epilepsy (e.g., focal cortical dysplasia), one might envisage a scenario where a single patient case study (with repeated observations from several slices) could provide insight into the potential efficacy of a novel anti-epileptic compound. Alternatively, one solution to low throughput issues and the heterogenous nature of samples would be to convene a number of laboratories working with human samples into a multi-center grouping. This approach would allow the “pooling” of samples and experimental studies and greatly increase productivity. This laboratory has made advances in this area by developing an in vitro system that allows electrophysiological recordings from multiple human brain slices at the same time. This system permits that each slice can be independently treated with pharmacological agents, maximizing the number of novel agents that could be tested in brain slices from pharmacoresistant patients. Other groups, using experimental refinements discovered in rodent brain slice studies (Buskila et al., 2014), have recently developed methods to prolong the longevity of acute human slice preparations to ca. 72 hours by treating the perfusing artificial CSF with UV light to prevent bacterial growth. This permits the use of more slices from each patient (Wickham et al., 2018).

There are numerous important practical considerations for the implementation of human brain tissue recordings. Primarily, such work is subject to the availability of tissue samples. Surgical resections are typically carried out as elective procedures at specialized hospital sites, and therefore the research lab intending to use the resected tissue must be near the hospital. Such sites typically generate regular specimens (for example resective surgical procedures at Beaumont Hospital, Dublin, generate approximately 5-10 brain specimens each month, with each specimen typically generating at least 15 brain slices for research).

It must also be considered that resected specimens are also required for neuropathological assessment as part of the clinical work-up. It is possible to divide specimens for research and pathology either in the surgical theatre or, if the pathologist is available to immediately dissect the tissue, this can be done in the pathology lab. In either case, detailed procedures must be established between surgeons, pathologists and researchers to ensure that research needs can be met with no impact on clinical procedures. The quality of the research specimen varies depending on how it is resected and handled. Typically, the most viable specimens for electrophysiological recordings are resected en bloc and submerged directly into a modified artificial cerebrospinal fluid for transport. However, for molecular and morphological analyses, it is sufficient to transport the specimen without any solutions.

Additionally, specific ethical approvals are required prior to any human tissue work, and informed consent must be obtained from each patient before their tissue can be used. Finally, interindividual differences such as age, sex and AED history may impact experimental observations and must be carefully considered.

To summarize, resected human tissue offers several key advantages over other model systems to study TLE (Tab. 1). It has the ultimate construct validity since it is the exact tissue of interest. It also exhibits strong predictive validity in terms of AED resistance and good face validity, although chemoclonovulsants may be required to trigger seizure-like activity in these specimens. However, relatively modest throughput, coupled with the practical considerations described, may represent drawbacks of this approach, some of which may be overcome using animal, iPSC-based and computational models.

4 Conclusion

Epilepsy is a serious neurological condition that has significant social and economic implications on a global scale. Despite many years of experimental research using animal models, a major shortcoming is the lack of drug efficacy for a significant proportion of patients. In this review, we have attempted to demonstrate that despite best efforts, numerous animal models fail to align with the clinical syndrome and recapitulate core features of the clinical disorder, i.e., drug refractory MTLE. We suggest that the use of live human epileptic tissue may provide improved clinical relevance by fulfilling the three key criteria of construct va-
lidity, face validity and predictive validity. In addition to important welfare issues, there is a clear scientific advantage to using epileptic human tissue. Detailed scientific studies of brain tissue from pharmacoresistant epilepsy patients allows a direct correlation between the patient’s phenotype and the underlying disease processes. This approach will increase the probability of identifying novel mechanisms that bring about pharmacoresistance in human epilepsy. This knowledge could be used to develop new medicines to provide therapeutic benefit to refractory epilepsy patients. Moreover, the use of epileptic human tissue will aid the ability to predict the effectiveness of novel AEDs that emanate from research and development pipelines. Indeed, the poor predictive nature of epilepsy animal models is being increasingly recognized by industry, academia, public and regulators. Whilst there is still much work to be done with regard to the reliability and relevance of epileptic human brain tissue, this approach will be beneficial for improving drug development and overcoming drug resistant epilepsy.

References

Akiyama, T., McCoy, B., Go, C. Y. et al. (2011). Focal resection of fast ripples on extraoperative intracranial EEG improves seizure outcome in pediatric epilepsy. Epilepsia 52, 1802-1811. doi:10.1111/j.1528-1167.2011.03199.x

Annegers, J. F., Hauser, W. A., Shirts, S. B. et al. (1987). Factors prognostic of unprovoked seizures after febrile convulsions. N Engl J Med 316, 493-498. doi:10.1056/nejm198702263160901

Aronica, E., Gorter, J. A., Ramkema, M. et al. (2004). Expression and cellular distribution of multidrug resistance-related proteins in the hippocampus of patients with mesial temporal lobe epilepsy. Epilepsia 45, 441-451. doi:10.1111/j.0013-9580.2004.57703.x

Aronica, E., Sisodiya, S. M. and Gorter, J. A. (2012). Cerebral expression of drug transporters in epilepsy. Adv Drug Deliv Rev 64, 919-929. doi:10.1016/j.addr.2011.11.008

Ben-Ari, Y. and Lagowski, J. (1978). Epileptogenic action of intra-amygdaloid injection of kainic acid. C R Acad Sci Hebd Seances Acad Sci D 287, 813-816 [Article in French]. http://www.ncbi.nlm.nih.gov/pubmed/103652

Ben-Ari, Y., Lagowska, J., Tremblay, E. et al. (1979). A new model of focal status epilepticus: Intra-amygdaloid application of kainic acid elicits repetitive secondarily generalized convulsive seizures. Brain Res 163, 176-179. doi:10.1016/0006-8993(79)90163-X

Ben-Ari, Y., Tremblay, E., Otterson, O. P. et al. (1980). The role of epileptic activity in hippocampal and ‘remote’ cerebral lesions induced by kainic acid. Brain Res 191, 79-97. doi:10.1016/0006-8993(80)90316-9

Benardo, L. S. (2003). Altered sodium channels underlie anticonvulsant drug insensitivity. Epilepsy Curr 3, 227-228. doi:10.1046/j.1535-7597.2003.03606.x

Benito-Kwiecinski, S. and Lancaster, M. A. (2019). Brain organoids: Human neurodevelopment in a dish. Cold Spring Harb Perspect Biol, a035709. doi:10.1101/cshperspect.a035709

Blümcke, I. (2009). Neuropathology of focal epilepsies: A critical review. Epilepsy Behav 15, 34-39. doi:10.1016/j.yebeh.2009.02.033

Bradt, C., Glien, M., Potschka, H. et al. (2013). International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: A task force report from the ILAE commission on diagnostic methods. Epilepsia 54, 1315-1329. doi:10.1111/epi.12220

Brandt, C., Volk, H. A. and Loscher, W. (2004). Striking differences in individual anticonvulsant response to phenobarbital in rats with spontaneous seizures after status epilepticus. Epilepsia 45, 1488-1497. doi:10.1111/j.1528-1167.2004.16904.x

Buskila, Y., Breen, P. P., Tapson, J. et al. (2014). Extending the viability of acute brain slices. Sci Rep 4, 4-10. doi:10.1038/srep05309

Cho, J. R., Joo, E. Y., Koo, D. L. et al. (2012). Clinical utility of interictal high-frequency oscillations recorded with subdural macroelectrodes in partial epilepsy. J Clin Neurol 8, 22. doi:10.3988/jcn.2012.8.1.22

Claes, L., Del-Favero, J., Ceulemans, B. et al. (2001). De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. Am J Hum Genet 68, 1327-1332. doi:10.1086/320609

Claes, L., Ceulemans, B., Audenaert, D. et al. (2003). De novo SCN1A mutations are a major cause of severe myoclonic epilepsy of infancy. Hum Mutat 21, 615-621. doi:10.1002/humu.10217

Covolan, L. and Mello, L. E. A. (2000). Temporal profile of neuronal injury following pilocarpine or kainic acid-induced status epilepticus. Epilepsia Res 39, 133-152. doi:10.1016/S0920-1211(99)00119-9

Cunningham, M. O., Roopun, A., Schofield, I. S. et al. (2012). Glissandi: Transient fast electrocorticographic oscillations of steadily increasing frequency, explained by temporally increasing gap junction conductance. Epilepsia 53, 1205-1214. doi:10.1111/j.1528-1167.2012.03530.x

Curia, G., Longo, D., Biagini, G. et al. (2008). The pilocarpine model of temporal lobe epilepsy. J Neurol Methods 172, 143-157. doi:10.1016/j.jneumeth.2008.04.019

de Tisi, J., Bell, G. S., Peacock, J. L. et al. (2011). The long-term outcome of adult epilepsy surgery, patterns of seizure remission, and relapse: A cohort study. Lancet 378, 1389-1395. doi:10.1016/S0140-6736(11)60890-8

Devinsky, O., Vezzani, A., Najjar, S. et al. (2013). Glia and epilepsy: Excitability and inflammation. Trends Neurosci 36, 174-184. doi:10.1016/j.tins.2012.11.008

Dodson, P. D. and Forsythe, I. D. (2004). Presynaptic K+ channels: Electrifying regulators of synaptic terminal excitability. Trends Neurosci 27, 210-217. doi:10.1016/j.tins.2004.02.012

Dubé, C., Richichi, C., Bender, R. A. et al. (2006). Temporal lobe epilepsy after experimental prolonged febrile seizures: Prospective analysis. Brain 129, 911-922. doi:10.1093/brain/awl018
Endele, S., Rosenberger, G., Geider, K. et al. (2010). Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat Genet 42, 1021-1026. doi:10.1038/ng.677

Engel, J. (1996). Introduction to temporal lobe epilepsy. Epilepsy Res 26, 141-150. doi:10.1016/s0920-1211(96)00043-5

Engel, J. (2001). Mesial temporal lobe epilepsy: What have we learned? Neurosci 7, 340-352. doi:10.1177/107385840100700410

Engel, J., Wiebe, S., French, J. et al. (2003). Practice parameter: Temporal lobe and localized neocortical resections for epilepsy. Epilepsia 44, 741-751. doi:10.1046/j.1528-1157.2003.48202.x

Engel, J. (2012). Early surgical therapy for drug-resistant temporal lobe epilepsy. JAMA 307, 922. doi:10.1001/jama.2012.220

Fang, M., Xi, Z.-Q., Wu, Y. et al. (2011). A new hypothesis of drug refractory epilepsy: Neural network hypothesis. Med Hypotheses 76, 871-876. doi:10.1016/j.mehy.2011.02.039

Flecknell, P. (2002). Replacement, reduction and refinement. ALTEX 19, 73-78. https://www.altex.org/index.php/altex/article/view/1106

French, E. D., Aldinio, C. and Schwarcz, R. (1982). Intrahippocampal kainic acid, seizures and local neuronal degeneration: Relationships assessed in unanesthetized rats. Neuroscience 7, 2525-2536. doi:10.1016/0306-4522(82)90212-3

French, J. A., Williamson, P. D., Thadani, V. M. et al. (1993). Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. Ann Neurol 34, 774-780. doi:10.1002/ana.40340604

Glen, M., Brandt, C., Potschka, H. et al. (2002). Effects of the novel antiepileptic drug levetiracetam on spontaneous recurrent seizures in the rat pilocarpine model of temporal lobe epilepsy. Epilepsia 43, 350-357. doi:10.1046/j.1528-1157.2002.18101.x

Goldberg, E. M. and Coulter, D. A. (2013). Mechanisms of epileptogenesis: A convergence on neural circuit dysfunction. Nat Rev Neurosci 14, 337-349. doi:10.1038/nn.3482

Gribkoff, V. K. and Kaczmarek, L. K. (2017). The need for new approaches in CNS drug discovery: Why drugs have failed, and what can be done to improve outcomes. Neuropharmacology 120, 11-19. doi:10.1016/j.neuropharm.2016.03.021

Grone, B. P. and Baraban, S. C. (2015). Animal models in epilepsy research: Legacies and new directions. Nat Neurosci 18, 339-343. doi:10.1038/nn.3934

Haegelen, C., Perucca, P., Chatillon, C.-E. et al. (2013). High-frequency oscillations, extent of surgical resection, and surgical outcome in drug-resistant focal epilepsy. Epilepsia 54, 848-857. doi:10.1111/epi.12075

Jacobs, J., Zijlmans, M., Zelmann, R. et al. (2010). High-frequency electroencephalographic oscillations correlate with outcome of epilepsy surgery. Ann Neurol 67, 209-220. doi:10.1002/ana.21847

Jandová, K., Pasler, D., Antonio, L. L. et al. (2006). Carbamazepine-resistance in the epileptic dentate gyrus of human hippocampal slices. Brain 129, 3290-3306. doi:10.1093/brain/awl218

Jones, R. S. G., da Silva, A. B., Whittaker, R. G. et al. (2016). Human brain slices for epilepsy research: Pitfalls, solutions and future challenges. J Neurosci Methods 260, 221-232. doi:10.1016/j.jneumeth.2015.09.021

Köhling, R., Lücke, A., Straub, H. et al. (1998). Spontaneous sharp waves in human neocortical slices excised from epileptic patients. Brain 121, 1073-1087. doi:10.1093/brain/121.6.1073

Kwan, P., Arzimanoglou, A., Berg, A. T. et al. (2009). Definition of drug resistant epilepsy: Consensus proposal by the ad hoc task force of the ILAE commission on therapeutic strategies. Epilepsia 51, 1069-1077. doi:10.1111/j.1528-1167.2009.02397.x

LaRoche, S. M. (2007). A new look at the second-generation antiepileptic drugs: A decade of experience. Neurologist 13, 133-139. doi:10.1097/nrl.0n0000256353.14257.7c

Lévesque, M. and Avoli, M. (2013). The kainic acid model of temporal lobe epilepsy. Neurosurg Rev 37, 2887-2899. doi:10.1007/s00062-013-1011-x

Lidster, K., Jefferys, J. G., Blümcke, I. et al. (2016). Opportunities for improving animal welfare in rodent models of epilepsy and seizures. J Neurosci Methods 260, 2-25. doi:10.1016/j.jneumeth.2015.09.007

Liou, J., Smith, E. H., Bateman, L. M. et al. (2020). A model for focal seizure onset, propagation, evolution, and progression. Elife 9, e50927. doi:10.7554/eLife.50927

Löschner, W. (1997). Animal models of intractable epilepsy. Prog Neurobiol 53, 239-258. doi:10.1016/S0301-0082(97)00035-X

Löschner, W. (2002). Animal models of drug-resistant epilepsy. Novartis Found Symp 243, 149-159; discussion 159-166, 180-185.

Löschner, W. (2011). Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure 20, 359-368. doi:10.1016/j.seizure.2011.01.003

Lytton, W. W. (2008). Computer modelling of epilepsy. Nat Rev Neurosci 9, 626-637. doi:10.1038/nrn2416

Maglóczy, Z. (2010). Sprouting in human temporal lobe epilepsy: Excitatory pathways and axons of interneurons. Epilepsy Res 89, 52-59. doi:10.1016/j.eplepsyres.2010.01.002

Marwick, K., Skehel, P., Hardingham, G. et al. (2015). Effect of a GRIN2A de novo mutation associated with epilepsy and intellectual disability on NMDA receptor currents and Mg2+ block in cultured primary cortical neurons. Lancet 385, Suppl 1, S65. doi:10.1016/S0140-6736(15)60380-4

Mathern, G. W., Pretorius, J. K. and Babb, T. L. (1995). Influence of the type of initial precipitating injury and at what age it occurs on course and outcome in patients with temporal lobe seizures. J Neurosurg 82, 220-227. doi:10.3171/jns.1995.82.2.0220

Mathern, G., Bertram, E., Babb, T. et al. (1997). In contrast to kindled seizures, the frequency of spontaneous epilepsy in the limbic status model correlates with greater aberrant fascia dentata excitatory and inhibitory axon sprouting, and increased staining for N-methyl-d-aspartate, AMPA and GABA_A rec. Neuroscience 77, 1003-1019. doi:10.1016/S0306-4522(96)00516-7
Mellanby, J., George, G., Robinson, A. et al. (1977). Epileptiform syndrome in rats produced by injecting tetanus toxin into the hippocampus. J Neural Neurosurg Psychiatry 40, 404-414. doi:10.1136/jnnp.40.4.404

Mello, L. E. A. M., Cavalheiro, E. A., Tan, A. M. et al. (1993). Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: Cell loss and mossy fiber sprouting. Epilepsia 34, 985-995. doi:10.1111/j.1528-1157.1993.tb02123.x

Millan, M. H., Chapman, A. G. and Meldrum, B. S. (1993). Extracellular amino acid levels in hippocampus during pilocarpine-induced seizures. Epilepsy Res 14, 139-148. doi:10.1016/0920-1211(93)90018-3

Miller, A. R., Hawkins, N. A., McCollom, C. E. et al. (2014). Mapping genetic modifiers of survival in a mouse model of Dravet syndrome. Genes Brain Behav 13, 163-172. doi:10.1111/gbb.12099

Moharaj, R. and Brodie, M. J. (2006). Diagnosing refractory epilepsy: Response to sequential treatment schedules. Eur J Neurol 13, 277-282. doi:10.1111/j.1468-1331.2006.01215.x

Mouri, G., Jimenez-Mateos, E., Engel, T. et al. (2008). Unilateral hippocampal CA3-predominant damage and short latency epileptogenesis after intra-amygdala microinjection of kainic acid in mice. Brain Res 1213, 140-151. doi:10.1016/j.brainres.2008.03.061

Niu, W. and Parent, J. M. (2020). Modeling genetic epilepsies in a dish. Dev Dyn 249, 56-75. doi:10.1002/dvdy.79

Oakley, J. C., Kalume, F., Yu, F. H. et al. (2009). Temperature- and age-dependent seizures in a mouse model of severe myoclonic epilepsy in infancy. Proc Natl Acad Sci 106, 3994-3999. doi:10.1073/pnas.0813330106

Ogawa, I., Miyamoto, H., Morita, N. et al. (2007). Na1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: A circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. J Neurosci 27, 5903-5914. doi:10.1523/JNEUROSCI.5270-06.2007

Okanishi, T., Akiyama, T., Tanaka, S.-I. et al. (2014). Intercital high frequency oscillations correlating with seizure outcome in patients with widespread epileptic networks in tuberous sclerosis complex. Epilepsia 55, 1602-1610. doi:10.1111/epi.12761

Putnam, T. J. and Merritt, H. H. (1937). Experimental determination of the anticonvulsant properties of some phenyl derivatives. Science 85, 525-526. doi:10.1126/science.85.2213.525

Quadrato, G., Nguyen, T., Macosko, E. Z. et al. (2017). Cell diversity and network dynamics in photosensitive human brain organoids. Nature 545, 48-53. doi:10.1038/nature22047

Quraishi, I. H., Stern, S., Mangan, K. P. et al. (2019). An epilepsy-associated KCNT1 mutation enhances excitability of human iPSC-derived neurons by increasing slack KNa currents. J Neurosci 39, 7438-7449. doi:10.1523/JNEUROSCI.1628-18.2019

Remy, S., Gabriel, S., Urban, B. W. et al. (2003). A novel mechanism underlying drug resistance in chronic epilepsy. Ann Neurol 53, 469-479. doi:10.1002/ana.10473

Roopun, A. K., Simonotto, J. D., Pierce, M. L. et al. (2010). A nonsynaptic mechanism underlying interictal discharges in human epileptic neocortex. Proc Natl Acad Sci 107, 338-343. doi:10.1073/pnas.0912652107

Salmi, M., Bolbos, R., Bauer, S. et al. (2018). Transient microstructural brain anomalies and epileptiform discharges in mice defective for epilepsy and language-related NMDA receptor subunit gene Grin2a. Epilepsia 59, 1919-1930. doi:10.1111/epi.14543

Sandow, N., Kim, S., Raue, C. et al. (2015). Drug resistance in cortical and hippocampal slices from resected tissue of epilepsy patients: No significant impact of P-glycoprotein and multidrug resistance-associated proteins. Front Neurol 6, 30. doi:10.3389/fneur.2015.00030

Schmidt, D. and Löscher, W. (2005). Drug resistance in epilepsy: Putative neurobiologic and clinical mechanisms. Epilepsia 46, 858-877. doi:10.1111/j.1528-1167.2005.54904.x

Sharma, A. K., Jordan, W. H., Reams, R. Y. et al. (2008). Temporal profile of clinical signs and histopathologic changes in an F-344 rat model of kainic acid-induced mesial temporal lobe epilepsy. Toxicol Pathol 36, 932-943. doi:10.1177/019266308326093

Shibley, H. and Smith, B. N. (2002). Pilocarpine-induced status epilepticus results in mossy fiber sprouting and spontaneous seizures in C57BL/6 and CD-1 mice. Epilepsia 49, 109-120. doi:10.1010/s0920-1211(02)00012-8

Simkin, D. and Kiskinis, E. (2018). Modeling pediatric epilepsy through iPSC-Based technologies. Epilepsy Curr 18, 240-245. doi:10.5698/1535-7597.18.4.240

Simon, A., Traub, R. D., Vladimirov, N. et al. (2014). Gap junction networks can generate both ripple-like and fast ripple-like oscillations. Eur J Neurosci 39, 46-60. doi:10.1111/ejn.12386

Sloviter, R. S. (2005). The neurobiology of temporal lobe epilepsy: Too much information, not enough knowledge. C R Biol 328, 143-153. doi:10.1016/j.crvi.2004.10.010

Sloviter, R. S. and Bumanglag, A. V. (2013). Defining “epileptogenesis” and identifying “antiepileptogenic targets” in animal models of acquired temporal lobe epilepsy is not as simple as it might seem. Neuropharmacology 69, 3-15. doi:10.1016/j.neuropharm.2012.01.022

Smart, S. L., Lopantsev, V., Zhang, C. L. et al. (1998). Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. Neuron 20, 809-819. doi:10.1016/s0896-6273(00)81018-1

Staba, R. J. (2013). Pathological oscillations in the pharmacoresistant epileptic brain. In Pharmacoresistance In Epilepsy (27-46). New York, NY, USA: Springer New York. doi:10.1007/978-1-4614-6464-8_3

Sterlini, B., Fruscone, F., Baldassari, S. et al. (2020). Progress of induced pluripotent stem cell technologies to understand genetic epilepsy. Int J Mol Sci 21, 482. doi:10.3390/ijms21020482

Sun, Y., Paşca, S. P., Portmann, T. et al. (2016). A deleterious Na1.1 mutation selectively impairs telencephalic inhibitory neurons derived from Dravet Syndrome patients. Elife 5, 1-26. doi:10.7554/eLife.13073

Sun, Y. and Dolmetsch, R. E. (2018). Investigating the therapeutic mechanism of cannabidiol in a human induced pluripotent stem cell (iPSC)-based model of Dravet syndrome.
Cold Spring Harb Symp Quant Biol 83, 185-191. doi:10.1101/sqb.2018.83.038174

Sutula, T., Xiao-Xian, H., Cavazos, J. et al. (1988). Synaptic reorganization in the hippocampus induced by abnormal functional activity. Science 239, 1147-1150. doi:10.1126/science.2449733

Tanouye, M. A., Ferrell, A. and Fujita, S. C. (1981). Abnormal action potentials associated with the Shaker complex locus of Drosophila. Proc Natl Acad Sci 78, 6548-6552. doi:10.1073/pnas.78.10.6548

Toyoda, I., Bower, M. R., Leyva, F. et al. (2013). Early activation of ventral hippocampus and subiculum during spontaneous seizures in a rat model of temporal lobe epilepsy. J Neurosci 33, 11100-11115. doi:10.1523/jneurosci.0472-13.2013

Traub, R. D., Cunningham, M. O. and Whittington, M. A. (2011). Chemical synaptic and gap junctional interactions between principal neurons: Partners in epileptogenesis. Neural Networks 24, 515-525. doi:10.1016/j.neunet.2010.11.007

Traub, R. D., Cunningham, M. O. and Whittington, M. A. (2014). What is a seizure network? Very fast oscillations at the interface between normal and epileptic brain. In H. Scharfman and P. Buckmaster (eds.), Issues in Clinical Epileptology: A View from the Bench. Advances in Experimental Medicine and Biology (71-80). Volume 813. Dordrecht, The Netherlands: Springer. doi:10.1007/978-94-017-8914-1_6

Tsai, M.-H., Chuang, Y.-C., Chang, H.-W. et al. (1998). Factors predictive of outcome in patients with de novo status epilepticus. QJM 102, 57-62. doi:10.1093/qjmed/hcn149

Turski, W. A., Cavalheiro, E. A., Schwarz, M. et al. (1983). Limbic seizures produced by pilocarpine in rats: Behavioural, electroencephalographic and neuropathological study. Behav Brain Res 9, 315-335. doi:10.1016/0166-4328(83)90136-5

UKHO (2019). Annual Statistics of Scientific Procedures on Living Animals Great Britain 2018.

Upton, N., Blackburn, T. P., Campbell, C. A. et al. (1997). Profile of SB-204269, a mechanistically novel anticonvulsant drug, in rat models of focal and generalized epileptic seizures. Br J Pharmacol 121, 1679-1686. doi:10.1038/sj.bjp.0701330

Traub, R. D., Cunningham, M. O. and Whittington, M. A. (2011). Chemical synaptic and gap junctional interactions between principal neurons: Partners in epileptogenesis. Neural Networks 24, 515-525. doi:10.1016/j.neunet.2010.11.007

Traub, R. D., Cunningham, M. O. and Whittington, M. A. (2014). What is a seizure network? Very fast oscillations at the interface between normal and epileptic brain. In H. Scharfman and P. Buckmaster (eds.), Issues in Clinical Epileptology: A View from the Bench. Advances in Experimental Medicine and Biology (71-80). Volume 813. Dordrecht, The Netherlands: Springer. doi:10.1007/978-94-017-8914-1_6

Tsai, M.-H., Chuang, Y.-C., Chang, H.-W. et al. (1998). Factors predictive of outcome in patients with de novo status epilepticus. QJM 102, 57-62. doi:10.1093/qjmed/hcn149

Turski, W. A., Cavalheiro, E. A., Schwarz, M. et al. (1983). Limbic seizures produced by pilocarpine in rats: Behavioural, electroencephalographic and neuropathological study. Behav Brain Res 9, 315-335. doi:10.1016/0166-4328(83)90136-5

UKHO (2019). Annual Statistics of Scientific Procedures on Living Animals Great Britain 2018.

Upton, N., Blackburn, T. P., Campbell, C. A. et al. (1997). Profile of SB-204269, a mechanistically novel anticonvulsant drug, in rat models of focal and generalized epileptic seizures. Br J Pharmacol 121, 1679-1686. doi:10.1038/sj.bjp.0701330

van der Staaay, F. J. (2006). Animal models of behavioral dysfunctions: Basic concepts and classifications, and an evaluation strategy. Brain Res Rev 52, 131-159. doi:10.1016/j.brainresrev.2006.01.006

van Gassen, K. L. I., Hessel, E. V. S., Ramakers, G. M. J. et al. (2008). Characterization of febrile seizures and febrile seizure susceptibility in mouse inbred strains. Genes Brain Behav 7, 578-586. doi:10.1111/j.1601-183X.2008.00393.x

VanLandingham, K. E., Heinz, E. R., Cavazos, J. E. et al. (1998). Magnetic resonance imaging evidence of hippocampal injury after prolonged focal febrile convulsions. Ann Neurol 43, 413-426. doi:10.1002/ana.410430403

Wang, J., Lin, Z. J., Liu, L. et al. (2017). Epilepsy-associated genes. Seizure 44, 11-20. doi:10.1016/j.seizure.2016.11.030

Wang, L., Wang, X., Yuan, J. et al. (2009). Nestin in the temporal neocortex of the intractable epilepsy patients. Neurochem Res 34, 574-580. doi:10.1007/s11064-008-9824-4

Wickham, J., Bröjdjegård, N. G., Vighagen, R. et al. (2018). Prolonged life of human acute hippocampal slices from temporal lobe epilepsy surgery. Sci Rep 8, 1-13. doi:10.1038/s41598-018-22554-9

Wiebe, S., Blume, W. T., Girvin, J. P. et al. (2001). Rationale and controlled trial of surgery for temporal-lobe epilepsy. N Engl J Med 345, 311-318. doi:10.1056/NEJM200108023450501

Wu, J. Y., Sankar, R., Lerner, J. T. et al. (2010). Removing interictal fast ripples on electrocorticography linked with seizure freedom in children. Neurology 75, 1686-1694. doi:10.1212/WNL.0b013e3181f2cd7d0

Yu, F. H., Mantegazza, M., Westenbroek, R. E. et al. (2006). Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat Neurosci 9, 1142-1149. doi:10.1038/nn1754

Zhang, C.-L., Messing, A. and Chiu, S. Y. (1999). Specific alteration of spontaneous GABAergic inhibition in cerebellar Purkinje cells in mice lacking the potassium channel K\(_{\text{v}}\)1.1. J Neurosci 19, 2852-2864. doi:10.1523/jneurosci.19-08-02852.1999

Zuberi, S. M., Eunson, L. H., Spauchus, A. et al. (1999). A novel mutation in the human voltage-gated potassium channel gene (K\(_{\text{v}}\)1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. Brain 122, 817-825. doi:10.1093/brain/122.5.817

Conflict of interest

The authors declare no conflict of interest.

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