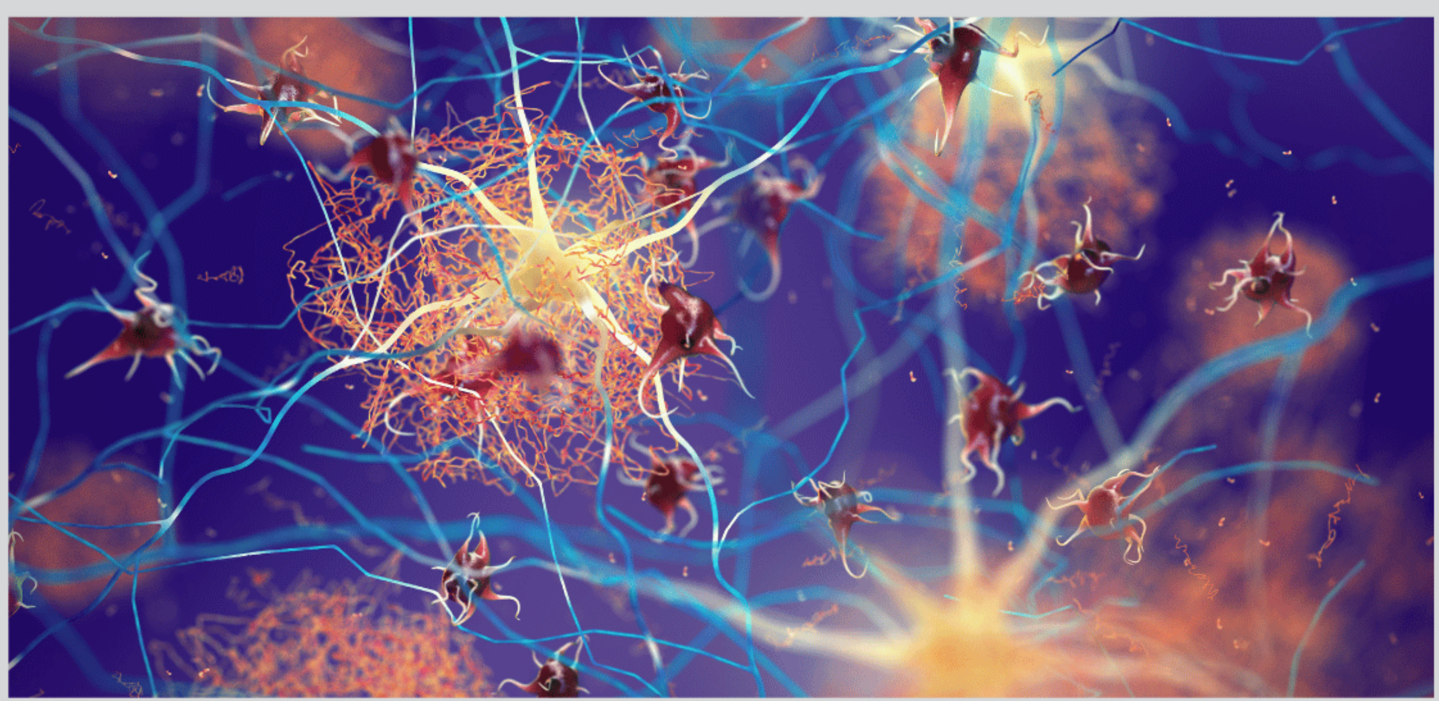
Initiated and funded by Eisai. Intended for UK/Ireland healthcare professionals.

Pathophysiological convergence of epilepsy and Alzheimer’s disease



Read about the common pathologies seen with both epilepsy and Alzheimer's disease, and learn more about clinical considerations

UK-NON-23-00089 Date of preparation June 2023.

Check for updates

Received: 6 June 2023 | Revised: 11 August 2023 | Accepted: 11 August 2023

DOI: 10.1111/epi.17744

Epilepsia™

SPECIAL REPORT

Can in vitro studies aid in the development and use of antiseizure therapies? A report of the ILAE/AES Joint Translational Task Force

Gareth Morris[1,2] | **Massimo Avoli**[3,4] | **Christophe Bernard**[5] | **Kate Connor**[6] | **Marco de Curtis**[7] | **Chris G. Dulla**[8] | **John G. R. Jefferys**[9,10] | **Caterina Psarropoulou**[11] | **Kevin J. Staley**[12] | **Mark O. Cunningham**[6]

[1]Division of Neuroscience, Faculty of Biology, Medicine and Health, School of Biological Sciences, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK

[2]Department of Neuroscience, Physiology and Pharmacology, University College London, London, UK

[3]Montreal Neurological Institute-Hospital and Departments of Neurology & Neurosurgery, McGill University, Montréal, Quebec, Canada

[4]Department of Physiology, McGill University, Montréal, Quebec, Canada

[5]Inserm, INS, Institut de Neurosciences des Systèmes, Aix Marseille Univ, Marseille, France

[6]Discipline of Physiology, School of Medicine, Trinity College Dublin, Dublin 2, Ireland

[7]Epilepsy Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

[8]Department of Neuroscience, Tufts University School of Medicine, Boston, Massachusetts, USA

[9]Department of Physiology, 2nd Medical School, Motol, Charles University, Prague, Czech Republic

[10]Department of Pharmacology, University of Oxford, Oxford, UK

[11]Laboratory of Animal and Human Physiology, Department of Biological Applications and Technology, Faculty of Health Sciences, University of Ioannina, Ioannina, Greece

[12]Neurology Department, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

Correspondence
Mark O. Cunningham, Discipline of Physiology, School of Medicine, Trinity College Dublin, Dublin 2, Ireland.
Email: mark.cunningham@tcd.ie

Funding information
Agentura Pro Zdravotnický Výzkum České Republiky, Grant/Award Number: NU21-04-00601; Associazione Paolo Zorzi for Neurosciences; Canadian Institutes of Health Research, Grant/Award Number: 8109, PJT153310 and PJT166178; Epilepsy Research UK, Grant/Award Number: F2102 and P1402; Excellence Initiative of Aix-Marseille University; Italian Health Ministry; National Institute of Neurological Disorders and Stroke, Grant/Award Number: R35NS116852;

Abstract

In vitro preparations (defined here as cultured cells, brain slices, and isolated whole brains) offer a variety of approaches to modeling various aspects of seizures and epilepsy. Such models are particularly amenable to the application of anti-seizure compounds, and consequently are a valuable tool to screen the mechanisms of epileptiform activity, mode of action of known anti-seizure medications (ASMs), and the potential efficacy of putative new anti-seizure compounds. Despite these applications, all disease models are a simplification of reality and are therefore subject to limitations. In this review, we summarize the main types of in vitro models that can be used in epilepsy research, describing key methodologies as well as notable advantages and disadvantages of each. We argue that a well-designed battery of in vitro models can form an effective and potentially high-throughput screening platform to predict the clinical usefulness of ASMs, and that in vitro models are particularly useful for interrogating mechanisms of

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2023 The Authors. *Epilepsia* published by Wiley Periodicals LLC on behalf of International League Against Epilepsy.

Royal Society, Grant/Award Number: RGS\R2\222326; Savoy Foundation; Science Foundation Ireland, Grant/Award Number: 16/RC/3948 and 20/FFP-P/8613

ASMs. To conclude, we offer several key recommendations that maximize the potential value of in vitro models in ASM screening. This includes the use of multiple in vitro tests that can complement each other, carefully combined with in vivo studies, the use of tissues from chronically epileptic (rather than naïve wild-type) animals, and the integration of human cell/tissue-derived preparations.

KEYWORDS

brain slice, cell culture, epilepsy, organotypic, seizure

1 | INTRODUCTION

1.1 | What is the goal of in vitro studies in epilepsy?

Epilepsy is one of the most common neurological diseases, affecting between 50[1] and 70[2] million people worldwide. Here, as part of the International League Against Epilepsy/American Epilepsy Society (ILAE/AES) Joint Translational Task Force, we discuss how in vitro preparations (defined as cultured cells, brain slices, and isolated whole brains, summarized in Table 1) can be used in the development of anti-seizure therapies. The underlying pathophysiology of epilepsy can be complex and multifaceted, and has been associated with numerous changes including cell death,[3] inflammation,[4] astrogliosis,[5] circuit re-wiring,[6] genetic variants,[7] and transcriptomic dysregulations.[8,9] In many cases, the etiology of epilepsy remains unknown. These changes may not only underlie seizure genesis or enhanced seizure probability but also participate in a range of co-morbidities associated with epilepsy[10] including memory loss, sleep disruption, and mood-related disorders, such as anxiety or depression. Frontline clinical treatment for epilepsy is with anti-seizure medications (ASMs). However, despite the availability of >30 ASMs,[11,12] approximately one third of people with epilepsy do not experience seizure freedom with these medications.[13] We define this phenomenon as drug-resistant epilepsy (DRE; also termed pharmacoresistant epilepsy). Novel ASMs must be made to treat patients with DRE, which justifies using in vitro studies as they allow a high throughput when screening compounds. Box 1 summarizes the key questions that can be addressed in vitro.

In vitro approaches offer a number of significant advantages over alternative methodologies for therapeutic screening (typically direct application of putative therapeutic compounds using in vivo animal models). First, the use of cell culture–based or acute slice seizure models circumvents the blood–brain barrier (BBB),[14] a key physiological limitation that can hinder in vivo drug screening by preventing potentially efficacious compounds from physically reaching the brain.[15] Because novel compounds can be applied directly to in vitro preparations, they provide a straightforward readout of efficacy that is not confounded by peripheral effects due to drug metabolism or the ability to cross the BBB. We note that this advantage can become a limitation, since potential anti-seizure therapies should cross the BBB. Moreover, the administration of novel compounds directly in vivo may raise ethical issues regarding animal welfare,[16] due to the possibility of unanticipated adverse effects. It is widely acknowledged that researchers must follow the principles of the 3Rs (reduce, refine, and replace) when conducting animal experiments.[16,17] In vitro models provide a powerful alternative, which addresses all three points to the greatest degree. In fact, statistical caution should be exercised to ensure that *enough* animals are used (for example, defining '*N*' as number of animals rather than number of cells or slices). This adheres to good experimental design and ensures sufficient statistical power to make valid inferences.

Key points

- In vitro models offer a variety of approaches for capturing various aspects of seizures and epilepsy.
- These models are particularly useful in screening mechanisms of anti-seizure compounds, and also provide higher throughput tools to screen efficacy.
- All models are simplifications of reality and each has inherent pros and cons. We propose, therefore, that best practice is to use a battery of in vitro models that can complement each other.
- A second recommendation is that in vitro studies into anti-seizure therapies should use tissues derived from chronically epileptic, rather than wild-type, animals.
- Finally, we recommend the integration of human tissue-based in vitro models into pre-clinical studies to develop anti-seizure therapies.

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 Overview of key advantages and disadvantages of each in vitro approach.

Type of model	Main advantages	Main disadvantages
Acute brain slice	• Maintenance of local neuronal connections and structure • Can be designed to retain wider connectivity between specific brain regions • Amenable to a variety of electrophysiological and optical techniques	• Wider brain connectivity is lost • Preparation is only viable for ~24 h • Slices are often prepared from non-epileptic brains
Whole brain preparation	• Expanded brain connectivity and structure is retained • Particularly suited to studying propagation of epileptic activity • Maintains blood–brain barrier function	• The intact blood–brain barrier may preclude application of some experimental compounds • Typically uses a non-epileptic brain
Organotypic slice culture	• Preparation can be maintained for several weeks • Some maintenance of local neuronal connections • Particularly suited to study genetic therapies, which need more time to take effect	• Slice cultures flatten and re-organize their connections over time • Slice cultures can generate spontaneous epileptiform activity, which may provide a moving baseline in assessing effects of anti-seizure medications
Isolated cells	• Favors high-throughput approach • Amenable to genetic manipulation to study effects of variants • Can be maintained long term	• Not realistic neurons • No realistic synaptic connectivity
Dissociated cell culture	• Use of real neurons (rather than cell lines) • Can be maintained long term • Can form synaptic connections	• Do not form realistic brain structures

BOX 1 Key goals when using in vitro models for pre-clinical research

Goal 1 – Mechanisms of epileptiform activity. A wide range of electrophysiological, biochemical and imaging techniques can be used in vitro to probe cellular and circuit mechanisms of epileptiform activity.

Goal 2 – Interrogating the mechanisms of existing anti-seizure medication. As in goal 1, in vitro preparations are amenable to a number of experimental techniques to probe functional mechanisms of brain networks. It is also easy and convenient to apply ASMs to in vitro preparations in order to interrogate their mechanism(s) of action.

Goal 3 – Screening new drugs. Similar to the approaches in goal 2, putative novel ASMs can be readily applied to in vitro preparations and screened for possible anti-seizure effects.

1.2 | Do in vitro studies bear any translational value?

It is often argued that animal models may not be relevant to human epilepsies because different species and different biological substrates are involved. Moreover, in vitro models could be considered less relevant, just because epilepsy is supposed to be a whole brain "network disease." We contend that any model is a good model if it has a predictive value, which is the *raison d'être* of a model. A model can only be validated a posteriori when the prediction generated by the model ultimately led to improved patient care. Thus any model is valid if it produces insights and predictions and does not just mimic features reported in people with epilepsy.

Any model is by construction a simplification of reality. Thus every model has intrinsic limitations due to the reductionist approach used to answer a given clinical/scientific question. In the following sections, we discuss different models and their main limitations.

1.3 | What do in vitro studies mean in the context of epilepsy?

Broadly, we define in vitro epilepsy models as those which use brain cells or networks of brain cells outside of a living organism. These systems must be capable of producing epileptiform activity, which may include seizure-like events, interictal-like patterns, status epilepticus (SE)–like activity, or cellular bursting discharges. Such activities can be evoked in a number of

ways, which can include the use of acute chemoconvulsants or electrical stimulation, or certain types of activity can occur spontaneously in ex vivo tissues taken from chronically epileptic rodents or human patients (Figure 1). A full discussion of different approaches to elicit activity sits outside the scope of this article, but readers are referred to previous reports from ILAE/AES Task Force groups.[18,19] There is a wide variety of in vitro systems available in experimental epilepsy research. Here, we will define each one, describe their key advantages and limitations, and provide examples of the types of activity that can be recorded in each type of preparation.

2 | TYPES OF IN VITRO PREPARATION

Here we provide an overview of the main types of in vitro models used in epilepsy research, and we discuss the key advantages and limitations of each. This information is summarized in Table 1 and Figure 1.

2.1 | Acute brain slices

Acute brain slice preparations refer to sections of brain tissue obtained immediately following euthanasia of an

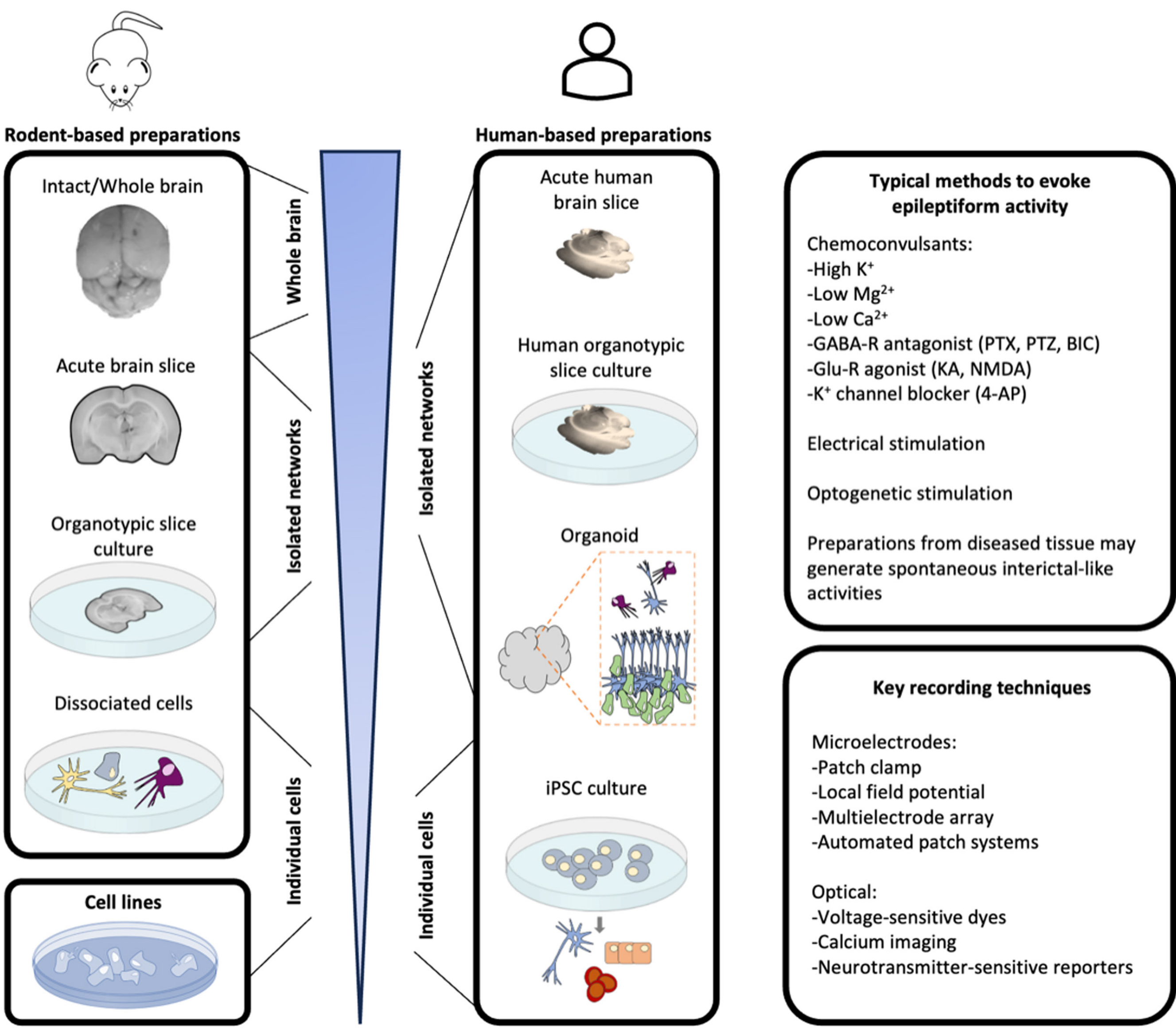


FIGURE 1 Rodent and human-based in vitro models offer a variety of approaches for capturing various aspects of seizures and epilepsy. Preparations range in scale may effectively capture whole networks and/or isolated neuronal systems. A well-designed combination of in vitro models can form effective and high-throughput screening platforms, facilitating the prediction of the clinical usefulness of ASMs and their mechanisms of action. 4-AP, 4-aminopyridine; ASM, anti-seizure medication; BIC, bicuculline; iPSC, induced pluripotent stem cell; KA, kainate; NMDA, N-methyl-D-aspartate; PTX, picrotoxin; PTZ, pentylenetetrazole.

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

animal (typically a mouse or rat) and kept alive artificially in a short-term (acute) storage chamber.[20] Brain slices typically remain viable for up to 12 h, meaning that they are amenable to roughly 1 day of experimentation (this timeframe can be extended to ~2 days when preparing slices from immature tissue under sterile conditions[21,22]). A notable advantage of this approach over neuronal cultures is that acute slices are obtained from intact brains and can include original local connections. Moreover, with careful choice of slice orientation and thickness, acute brain slices are able to retain connections between different regions of the brain, which may be relevant to the propagation of epileptiform activity. For example, hippocampal slices can be obtained with retained connectivity to and from key related structures, including the entorhinal[23–26] and perirhinal cortices[24] and the amygdala.[24]

In cases where acute brain slices are obtained from naïve (non-epileptic) rodents, epileptiform activity does not occur spontaneously and is typically induced by altering the composition of cations within the artificial cerebrospinal fluid (aCSF) perfusate (e.g., zero magnesium, elevated potassium, or reduced magnesium/elevated potassium combination),[27–31] or using chemoconvulsants (e.g., 4-aminopyridine, picrotoxin, bicuculline, or kainate); these experimental procedures alter excitation[32] and/or inhibition[33–35] within the brain slice. These methods have parallels with symptomatic seizures in the clinic, where abnormal levels of cations or exposure to chemoconvulsants can also trigger acute seizures in humans. In brain slices, epileptiform activity can also be generated by electrical stimulation.[36] Consideration should be given to the anatomic gradients of excitability within regions of interest (e.g., hippocampus and entorhinal cortex) and a careful correlation of seizure activity with slices obtained from along the septo-temporal or dorso-ventral axis. In vitro brain-slice studies in the entorhinal cortex[37] and hippocampus[38,39] have shown significant differences in ictal and inter-ictal initiation dependent on the precise anatomic location from which the slices were obtained. These differences in epileptiform activity across the respective axes are likely to reflect alterations in both intrinsic neuronal features and inhibitory networks.

Acute brain slices have been used to demonstrate the influence of metabolic pathways for seizure-like activity. Exposure to mitochondrial toxins (rotenone, potassium cyanide) and an aconitase inhibitor (fluorocitrate) produced repetitive inter-ictal discharges in hippocampal networks.[40] In contrast, perturbation of a specific element of neuronal metabolism, mitochondrial dihydroorotate dehydrogenase, demonstrated reductions in neuronal and synaptic excitability in vitro.[41] Alterations in cellular metabolism are emerging as an important driver in both acquired (for e.g., hypoxic–ischemic, traumatic brain injury, vascular dysfunction, SE, and brain tumor related epilepsy) and particular genetic epilepsies (for e.g., *SLC135A*, *SLC2A1*, *POLG1*, myoclonic epilepsy with ragged red fibers, mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes, and *ALDH5A1*). Further work utilizing in vitro models in this specific area should indeed provide additional insights into the pathophysiology of these conditions.

Epileptiform activity recorded in brain slices is macroscopically similar to interictal discharges and seizures recorded in patients,[42] and therefore slice models have provided a rapid and realistic preparations to screen putative novel therapeutic compounds.[32] By studying dynamic cellular and network changes occurring in the slice between chemoconvulsant application and the onset of seizure-like events in naïve slices, it may be possible to interrogate mechanisms of ictogenesis, seizure onset, and seizure termination (for example[43]). However, as mentioned above, epilepsy is associated with alterations in brain circuits, which are not present in naïve control slices. For example, fast ripple oscillations, which may be a pathological biomarker of epileptic networks, can be seen in ex vivo tissues from chronically epileptic rats in response to elevated extracellular potassium, but the same phenomenon is usually not observed using control brain slices.[29] Moreover, ex vivo slices obtained from rodents with epilepsy have been demonstrated to generate spontaneous interictal-like activity[44] (although ictal-like activity often still needs to be evoked with chemoconvulsants[29,44,45] and/or electrical stimulation[46]). There is always the possibility that a compound validated in slices from non-epileptic animals may not work in slices from experimental models of epilepsy. Although more time-consuming and costly, it may be preferable to test compounds in acute brain slices from rodent in vivo models of chronic acquired[29] and genetic epilepsies.[47] Using slices from experimental models of epilepsy raises further challenges. A compound may be efficient in one model/species/strain and not in another. Ideally, compounds should be tested in the seizure-onset zone. Although studies performed in acute human slices (see below) allow direct measurements in the epileptogenic zone obtained after neurosurgery,[48–50] the epileptogenic zone is rarely identified in experimental models before performing slice experiments. This would require using the same strategy as in patients with DRE: performing multisite recordings to estimate the onset zone and then extract the brain and make slices that include the onset zone. We are aware of only two studies in which multisite recordings have been performed, in pilocarpine-treated rats[51] and in kainic acid–treated mice[52] to identify the onset zone. Although it increases both the complexity and the cost of the experiment, this approach is technically possible. Such slices likely offer greater insight into

human epilepsy, and a key recommendation of our report is the use of ex vivo brain slices obtained from epileptic rodents, where possible (Box 2).

BOX 2 Key recommendations when using in vitro models for pre-clinical research

- Given that each in vitro preparation has inherent advantages and limitations, a battery of tests must be chosen carefully to assess mechanisms and efficacy in various disease models.
- Although in vitro preparations serve as a higher throughput initial screening tool, subsequent use of in vivo models may help to consolidate evidence of pre-clinical efficacy.
- Where possible, in vitro studies with rodent tissue should use brains from chronically epileptic animals, rather than non-diseased control tissues. This better captures the epileptic brain state, which is pre-disposed to spontaneous seizures and may provide a more valid screening tool.
- Where possible, animal-based preparations should be complemented with human-based tissues in pre-clinical pipelines. This provides valuable translational evidence of efficacy in human-derived neurons or brain tissues, and provides evidence against potential species-specific effects.

2.2 | Intact and whole brain preparations

Intact in vitro preparations are those where the whole intact structure and connections of specific regions (e.g., hippocampus) is removed and maintained in vitro. This type of preparation offers the researcher an expanded anatomic connectivity relative to slice preparations and the ability to stimulate connected regions or pathways. These intact preparations also have the advantage that they maintain anatomic gradients within the isolated structures. The in vitro intact hippocampal[53] and cortico-hippocampal[54] formations have been used to study the induction and propagation of epileptiform activity, and also for therapeutic screening of ASMs.[55]

The isolated guinea pig brain was developed initially[56] for the purposes of studying multi-synaptic connections in an intact whole brain. This preparation is advantageous over other in vitro preparations, as it maintains long-range functional connectivity, BBB function, and neurovascular interactions, and the intact vasculature can be used to ensure robust supply of oxygenated aCSF to all brain regions (this is an advantage over brain slices in which brain activity is highly influenced by the amount of oxygen provided by the perfusate[29,57]). Therefore, robust seizure activity can be studied simultaneously in different areas and in both hemispheres using conventional electrophysiological approaches from single cells to neuronal networks. Similar to brain slices, epileptiform activity has to be evoked by pharmacological manipulations. In addition, other techniques such as ion sensitive electrodes (pH, K^+), voltage sensitive dyes,[58,59] intrinsic optical imaging,[59,60] and calcium imaging[61,62] commonly used in slices can also be applied in this preparation. Another advantage of the isolated whole brain preparation over in vitro brain slice approaches is the presence of an anatomically intact neurovascular unit.[63,64] However, the effects of vasodilatation/vasoconstriction in the absence of the pulsatile action of heart beats, and how it reacts to the presence of non-physiological oxygen concentration (95% instead of 20%), remain to be assessed. Dysfunction of the neurovascular unit is associated with seizures and epilepsy[65]; in the isolated brain the BBB can be manipulated experimentally[66] in order to probe its impact on epilepsy in the absence of peripheral influences, which may confound in vivo studies. This could be developed further by using the isolated brain from an already epileptic guinea pig, which may recapitulate BBB dysfunction seen in the human condition. In the context of drug-resistant seizures, neuroinflammatory changes and BBB alterations have been implicated in the seizure pathophysiology. The ability of this particular in vitro model to retain functionally viable and interactive neuronal, glial, neurovascular,[67] and neuroinflammatory[68] elements of the system makes it useful for testing hypotheses and novel therapeutic avenues that involve neurovascular[69] and/or neuroinflammatory aspects. Finally, the isolated brain preparation can be used to evaluate BBB permeability of ASM and brain active molecules.[70] One limitation of this approach is the relative difficulty in visualizing and accessing specific cells, particularly in structures deep within the brain such as the hippocampus, which may be more readily targeted for recording in slice preparations from specific brain regions.

2.3 | Organotypic slice cultures

Organotypic slice cultures are first obtained from acutely prepared brain slices, and then maintained in culture for longer-term use.[71–73] Slices prepared in this way can be kept for several weeks, vastly prolonging the lifetime of the tissue when compared with acute approaches. This timescale is the most notable advantage of organotypic slice cultures. Certain experimental interventions (e.g., antisense oligonucleotides [ASOs][74] or viral vectors[75,76])

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

may require timescales on the order of days to weeks to mediate their effects. This is longer than the feasible viability of acute slice preparations. A further property (and a technical necessity) of organotypic slice cultures is that they are derived from young (typically <postnatal day 10) rodents, and that the neurons and circuits within these slices continue to mature in culture. Therefore, another possible application of this technique is to probe how ASM mechanisms and efficacy change with neurodevelopment, which could have particular relevance to the treatment of childhood epilepsies. Although organotypic slice cultures retain some advantages of acute slices, there are also drawbacks to this approach. Over time in culture, organotypic slices naturally flatten as they adhere to their semi-permeable membrane support[73] and re-organize their synaptic connections.[73]

Of interest, cultures from non-epileptic brains eventually generate spontaneous interictal-like activity and seizures-like events,[77] offering insights into the reorganization process that is considered part of in vivo epileptogenesis. This has been exploited as a model of acquired epilepsy after brain injury.[78] In drug-screening studies, organotypic slice responses have been validated using in vivo acquired epilepsy models.[79]

2.4 | Isolated cells

A high-throughput in vitro approach uses isolated cultured cell lines, such as HEK293 or neuro2A cells, and automated patch clamp systems.[80] At the time of writing, leading high-throughput screening systems are able to record from 384 cells simultaneously.[80] By transfecting ion channels into these isolated cells, researchers can quickly record the activity of these channels in large numbers of cells. In epilepsy research, this permits the rapid phenotypic interrogation of genetic variants in relevant ion channels.[81] Another application is high-throughput screening of the effects of a large number of compounds on specific ion channels.[82] Isolated cells also offer technical advantages: they have superior space clamp properties compared with primary neurons[83] and they are amenable to single-channel recordings.[84] The main drawback of such approaches is that they are far from being representative of realistic brain cell or circuit structures. Therefore, any findings at the channel level discovered using this method must be validated using more realistic models in order to infer their effects on epileptiform activities, which are network-driven events.

It is important to note that the presence of a genetic variant is not necessarily responsible for ictogenesis, although it may produce epilepsy.[85] Furthermore, the presence of a variant does not necessarily lead to modification of firing behavior in neurons—a form of degeneracy, which in general refers to the idea that multiple component parameter values in a system can give rise to the same output.[86]

2.5 | Dissociated cell cultures

A different approach to cellular models is the use of primary dissociated cultures.[87] Typically, brains are dissected from rodent pups and their cells are dissociated, plated, and maintained in culture. Cultures can be purely neuronal, or they can contain mixtures of neurons and glia. A clear advantage over automated screening methods is the use of real neurons, as opposed to cell-line systems. Therefore, dissociated cultures are capable of producing realistic epileptiform bursting activity at the single-cell level.[88,89] In silico modeling has been used to link activity in single neurons with different epileptiform activities.[90] Moreover, dissociated neurons are capable of forming synaptic connections and networks in culture, and can be plated on to microelectrode arrays (MEAs) to simultaneously monitor the activity of multiple neurons. MEA recordings have also been also used to analyze the dynamics of synchronous epileptiform activities in brain slices.[91,92]

Although primary cell cultures are less realistic than the microcircuits preserved in slice preparations, they do facilitate the study of synaptic connectivity and network activity. One technical advance used a modified dynamic clamp approach to elicit realistic single-neuron activity, recorded from a seizing brain slice, into a pharmacologically isolated dissociated neuron.[93] These studies exploited this model to test the effects of anti-seizure compounds on epileptiform bursts at the cellular level, revealing novel mechanisms of the ASM carbamazepine. As with other methods, dissociated cells can be prepared from in vivo genetic epilepsy models, retaining the underlying pathological gene variants for in vitro studies. Glioneuronal cultures[94,95] exposed to zero magnesium CSF and imaging techniques have been used to assess reactive oxygen species (ROS) pathways during seizure activity—an example of interrogating the impact of metabolic pathways on epileptiform events.

3 | TRANSLATIONAL CAPABILITIES OF IN VITRO PREPARATIONS

3.1 | Improved translation of in vitro models—Use of human-based preparations

Our discussion, so far, has focused mainly on the use of animal-derived tissues for in vitro epileptic seizure

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

models. This raises a key concern in the context of human ASMs. Although rodent brains provide reasonable representations of human brain circuitry, there are clearly substantial differences between the two that render direct comparisons difficult to interpret. Solutions to this aspect lie in the use of human-derived tissues in vitro to model seizures and epileptiform activity. At the cellular level, distinct cell types including neurons and glia can be derived from induced pluripotent stem cells (iPSCs).[96–99] Briefly, human somatic cells such as skin fibroblasts or peripheral blood mononuclear cells are harvested from donated patient samples and reverted to an induced pluripotent state via overexpression of a series of four transcription factors (OCT4, SOX2, KLF4, MYC; known as Yamanaka reprogramming factors[99]). These iPSCs can then be differentiated into the desired cell type. A key advantage of iPSCs is that they retain the human genome of the original cell (assuming no somatic mutations). This is particularly advantageous in the context of genetic diseases, where exact genetic variants are preserved. iPSC-derived cells are then grown in culture and have applications similar to dissociated rodent neuronal cultures, discussed above. These cells can also be cultured in three-dimensional scaffolds to grow realistic brain organoids. Using this method, it is possible to recapitulate complex brain anatomy, including cortical columns.[100] Organoids may offer a unique model to study the impact of genetic variants on brain development, which is of particular relevance to epilepsies associated with brain malformation. Organoids develop at approximately the same rate as the human brain in utero, so the maturation of organoids to the point where synchronous network activity is detected involves several months of culture.[101] Indeed, brain organoids have been derived from patients with developmental epileptic encephalopathies including Rett syndrome[102] and variants of the WW domain-containing oxidoreductase gene.[103] These organoids recapitulated key disease phenotypes such as single-cell transcriptomic dysregulation and epileptiform network activity. More recently, brain organoids were used in the preclinical development of a new gene therapy strategy,[104] emphasizing their ability to enhance translational research in epilepsy. Despite the human derivation of iPSCs and organoids, some doubts remain. Notably, it is unclear how accurately iPSCs in culture mature and thus whether they represent functional human neurons. In the case of organoids, these are devoid of external stimuli, which might impact the natural physiological development of real human brain systems. Therefore it should be considered that organoids might not fully capture all aspects of human neurodevelopment.

Another solution is offered by the use of surgically resected human brain tissue.[48,105] For some patients with focal DRE, surgical removal of their seizure-onset zone is a viable clinical option to reduce or remove their seizure burden.[106] With informed patient consent, this tissue can be collected and processed in the same way as rodent brain tissues. This is arguably the ideal model of DRE—the exact seizure-onset zone that could not be treated with ASMs. Human brain specimens can be sectioned for acute slice recordings and typically have longer viability than rodent slices. An adapted slice storage method can be used to extend their viability for up to 72 h,[107] permitting the screening of ASO-based therapies in human brain.[108] For longer term use, including the application of viral vectors, human brain slices can be maintained in organotypic culture.[109,110] This approach has been extended recently to the study of the developing human brain.[111] Resected human slices do suffer some disadvantages. Patient donors and tissue samples are highly heterogeneous with key variables including patient age, sex, ASM history as well as the anatomic location of the seizure-onset zone and the viability of the resected tissue. Moreover, heterogeneity of slices from within samples in terms of their pharmacological response to anti-seizure drug application and effect on electrophysiological recordings has been reported.[112] There is also no direct control for such tissues, although non-epileptic cortical regions, resected for access to deeper structures, are often used. In light of the questions associated with the interpretation of the epileptic human brain slice models, it should be mentioned that such slices often do not exhibit spontaneous seizures but do in some cases generate spontaneous inter-ictal activity,[112–117] which may mainly mirror γ-aminobutyric acid (GABA)ergic processes.

3.2 | How well do in vitro assays predict in vivo and clinical usefulness of ASMs?

Several in vitro models have been used to test ASM effects with a view to translating findings from preclinical testing to human clinical trials. In addition to reducing animal use, this approach could also aid with guiding drug dosing in trials. Testing ASM efficacy in vitro also allows a clean dissection of seizure modifications in the absence of potential pharmacokinetic complications that arise from in vivo systems approaches. Furthermore, the use of in vitro models facilitates quick screening of drugs individually or in combination.[118] This combinational approach may be useful to help gain better a quantitative understanding of "rational polytherapy," which would be more complex in vivo.

One in vitro model that has been used for ASM screening is the "Mg^{2+}-free" or "zero Mg^{2+}" model. Removal of magnesium from the bathing medium results in epileptiform events in cortical and hippocampal slices.[30] However,

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

these events evolve over time to become short recurrent discharges (termed late recurrent discharges [LRDs]). The evolution of washout of Mg^{2+} has been shown to reduce GABA-mediated inhibition[119] and the LRDs are likely to reflect disinhibition within neuronal networks. Of interest in the context of drug screening, the LRDs are frequently pharmacoresistant to standard ASMs[120] such as phenytoin, phenobarbital, and midazolam. The insensitivity to benzodiazepines has led to the suggestion that zero Mg^{2+} LRDs may be considered an in vitro model of SE. Recent work using organotypic hippocampal slices[121] has demonstrated that persistent SE-like activity is associated with a reduction in GABA A receptor conductance and chloride extrusion capability. Alternatively, the LRDs may reflect interictal activity, which can also persist in the presence of ASMs.[122] In contrast, the potassium channel blocker 4-aminopyridine (4-AP) produces stable occurrence of seizure-like (ictal) events and associated inter-ictal events in several cortical (entorhinal, perirhinal, amygdala) and hippocampal regions of brain slices.[122,123] The 4-AP model has been used to capture the efficacy of newer-generation ASMs including lacosamide, zonisamide, and levetiracetam,[124] as well as classic ASMs such as CBZ, topiramate (TPM), and valproate (VPA).[122] The elevated potassium (high K^+) hippocampal slice model[28] has been used to screen the efficacy of the ASMs lamotrigine, phenytoin, and valproate, both individually and in combination.[118] This exemplifies the use of in vitro models to interrogate polytherapy. Many people with drug-resistant seizures experience polypharmacy, and such experiments are therefore key to fully reflect the setting in the epilepsy clinic. Finally, it should be noted that the simplicity of in vitro models may also be a drawback. Putative ASMs may be efficacious in vitro but not in vivo,[125] presumably due to peripheral metabolism and the BBB in vivo. They may also have different effects in ex vivo tissue from chronically epileptic animals, which likely have different mechanisms of ictogenesis when compared with naïve tissues in which we induce seizures typically by modifying the extracellular environment.

3.3 | Does the use of in vitro models in experimental studies aid in identifying ASM mechanisms?

A key advantage of in vitro preparations for mechanistic drug discovery is the ability to visualize brain activity at the cellular level. A fundamental technique in this regard is patch-clamp electrophysiology,[84] allowing the researcher to precisely record the activity of individual identified cells and comparing their activity in the presence and absence of ASMs. Current clamp recordings can reveal changes in passive biophysical properties such as resting membrane potential and input resistance, as well as active firing properties including action potential waveforms and firing rates. Voltage clamp recordings can also be used to either monitor inputs to the individual neuron or to isolate the currents from specific voltage-gated ion channels. These approaches can be combined to demonstrate the effects of ASMs on individual biophysical processes, which ultimately change the properties of individual neurons and in turn neuronal networks. Using extracellular recording of slice seizure models combined with simultaneous patch clamp of individual neurons in the network, it is possible to resolve both single cell and network level activity at the same time, and so to infer the relationship between the two.[29] Another advantage of in vitro approaches for understanding drug mechanisms is the ease and speed with which ASMs can be applied to preparations. This allows the researcher to record a baseline measurement (without ASM), rapidly apply the ASM to observe its effect, and then wash it out. This is less straightforward with highly lipid-soluble agents such as cannabinoids, which progressively partition into cellular membranes during exposure and wash out very gradually.[126] However, for most drugs, this means that it is straightforward to assess the activity of the same cells or networks in the presence and absence of a well-controlled concentration of ASM. This is extremely challenging in vivo, where ASMs must typically be applied systemically. This means that ASMs take a long time to reach the brain, and that the researcher has much less control over the active concentration of the ASM reaching the brain.

It is important to understand the mechanisms of ASMs in order to identify their possible indications or contraindications in the epilepsy clinic. For example, in vitro studies show that ASMs including CBZ and phenytoin inhibit voltage-gated sodium channel function as their main anti-seizure mechanism.[127] This mechanism means that they are strong frontline ASMs, but also contraindicated their use in epilepsies associated with impaired firing of inhibitory neurons, for example Dravet syndrome.[128] There are several notable examples in drug discovery where in vitro interrogation has revealed unexpected mechanisms of ASMs, often different from the mechanism intended in their design. For example, gabapentin and pregabalin are rationally designed analogues of GABA, which were intended to treat epilepsy by increasing GABA availability and so network inhibition in the brain. However, it was later discovered that despite their design, these ASMs have no clear impact on the GABAergic system, instead mediating their anti-seizure effects by targeting voltage-gated calcium channels.

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

4 | WHAT COMES NEXT IN TERMS OF USING IN VITRO STUDIES FOR TRANSLATIONAL EPILEPSY RESEARCH?

4.1 | Developments and opportunities using human-derived in vitro models

A key limitation of most classical in vitro models is that they are typically derived from animal tissues and do not reflect the complexity of human neurons and brain networks (for example[129,130]). Therefore, antiseizure molecules that are efficacious in animal-based models may not address the full molecular, biophysical, genetic, and anatomic complexity of human seizures and, ultimately, may not work in humans the same way they do in animal-based models. Typically, this may not be evident until a time-consuming and costly pre-clinical pipeline has been completed. This may include rodent in vivo studies, followed by Good Laboratory Practice (GLP) grade studies in another species (possibly dog or non-human primate) and then even first-in-human trials, before lack of translation between animal and human-derived tissues becomes apparent. This is a hugely costly strategy in terms of time, money, and also ethically, owing to the animals used. Recent advances in the use of human-derived tissues for translational epilepsy research are invaluable to bridge this gap[105,108,109,131] and to provide relatively cheap and fast human-based screens of new therapeutics, before they begin further pre-clinical validation. For small molecules, acutely resected human epileptic tissue can be used as an in vitro seizure screen. For approaches with slower mechanisms of action, such as nucleic acid or virally-delivered therapies, these slices can be maintained in organotypic cultures.[110] For approaches targeting the genetic epilepsies, advances in iPSC and gene-editing technology, as well as brain organoids, provide strong platforms that clearly mirror the human genetic pathology. Of course these strategies have limitations (they are not whole brain preparations) and they must be used strategically in parallel with in vivo animal models, or with potentially higher throughput animal-based in vitro models. Nevertheless, human-derived in vitro models can provide an early screen of drug efficacy in human brain and should be integrated into pre-clinical pipelines at an early stage.

4.2 | In vitro optical approaches to translational epilepsy research

Optical technologies are rapidly advancing and provide key new tools in translational drug discovery for epilepsy. Broadly, optical approaches can be used to modulate neuronal activity (optogenetics[132]) and/or as a readout of neural activity.[133] The combination of these techniques is referred to as "all-optical" neurophysiology.[134] This has certain advantages over electrophysiological techniques. For example, when using light as a readout it is straightforward to measure the cellular activity of many neurons simultaneously. Optical tools to read out neuronal activity can include voltage indicators,[133] calcium sensors,[135] or fluorescent reporters, which are sensitive to neurotransmitters such as glutamate[136] and GABA.[137] This is much more laborious using electrophysiology and, in the case of patch-clamp approaches, it is more technically challenging and limited by the number of micropipettes that can be physically placed into the preparation. Indeed, closed-loop systems have been developed that use optogenetic mediators to manipulate circuit-level excitability in response to input from electrically recorded network activity.[138,139] The authors of the study[138] argue that such an approach provides benefit for mechanistic studies and also offers translational benefit, although others have noted that challenges may remain with delivery of light to deep brain structures in a clinical setting.[140] This concept was developed initially using in vitro in brain slice preparations,[138,139] demonstrating the utility of in vitro approaches for developing optogenetic-based therapies.

We believe that the application of these rapidly advancing technologies to human tissue–based in vitro preparations will offer substantial translational and mechanistic insights into epilepsy. Typically the use of these optical technologies requires genetic transduction of fluorescent sensors into neurons. Therefore, transduction into human brain preparations would either require the use of fast-acting viral vectors (which have translational limitations), or human brain organotypic slice cultures, which permit viral expression using adeno-associated virus (AAV).[109]

5 | CONCLUSIONS

A variety of in vitro preparations can be used to study epilepsy and anti-seizure compounds. These preparations range from individual cells through to brain slices and whole brain preparations. In vitro preparations are amenable to a number of recording techniques, both electrophysiological and optical, at the single cell and network levels. The increasing use of human cell- and tissue-based in vitro techniques can enhance the clinical relevance of the approaches. Given the relative convenience of these preparations, different in vitro techniques can be combined into a battery of complementary approaches to enhance their utility in pre-clinical epilepsy research. In summary, we propose a series of recommendations (Box 2) to guide the careful use of in vitro seizure models

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

to provide detailed mechanistic and therapeutic insights in the development of new anti-seizure therapies.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the article. GM and MOC wrote the initial article. All authors edited and approved the final article.

ACKNOWLEDGEMENTS

None. Open access funding provided by IReL.

FUNDING INFORMATION

This report was written by experts selected by the International League Against Epilepsy (ILAE) and the American Epilepsy Socety (AES) and was approved for publication by the ILAE and AES. Opinions expressed by the authors, however, do not necessarily represent the policy or position of the ILAE or AES. GM is supported by a Fellowship from Epilepsy Research UK (Morris F2102) and by the Royal Society (RGS\R2\222 326). MA acknowledges the Canadian Institutes of Health Research (CIHR, Operating Grant 8109, PJT153310 and PJT166178) and the Savoy Foundation. CB received funding from Excellence Initiative of Aix-Marseille University - A*Midex (project ION), a French "Investissements d'Avenir" programme. MdC received funding from Associazione Paolo Zorzi for Neurosciences and from the Italian Health Ministry. JGRJ is supported by Czech Health Research Council, Ministry of Health of the Czech Republic grant NU21-04-00601, and the James Lewis Foundation through Epilepsy Research UK (P1402). KJS acknowledges funding from the National Institutes of Health, National Institute of Neurological Disorders and Stroke R35NS116852. This publication has emanated from research supported in part by research grants from Science Foundation Ireland (SFI) under Grant Number 20/FFP-P/8613 (Frontier for the Future project award) and Grant Number 16/RC/3948 and co-funded under the European Regional Development Fund and by FutureNeuro industry partners.

CONFLICT OF INTEREST STATEMENT

The authors acknowledge the financial support of the International League Against Epilepsy (ILAE) and the American Epilepsy Society (AES) for the ILAE/AES Joint Translational Task Force activities. MdC received occasional support from UCB and Angelini Pharma. CGD has a sponsored research agreement and is a consultant for BridgeBio Pharmaceuticals. All other authors have no conflicts of interest to declare.

ORCID

Gareth Morris https://orcid.org/0000-0003-2469-5102
Massimo Avoli https://orcid.org/0000-0003-1876-9638
Christophe Bernard https://orcid.org/0000-0003-3014-1966
Kate Connor https://orcid.org/0000-0001-5044-1240
Marco de Curtis https://orcid.org/0000-0001-7443-6737
Chris G. Dulla https://orcid.org/0000-0002-6560-6535
John G. R. Jefferys https://orcid.org/0000-0003-0106-4412
Caterina Psarropoulou https://orcid.org/0000-0002-1929-6951
Kevin J. Staley https://orcid.org/0000-0002-3072-518X
Mark O. Cunningham https://orcid.org/0000-0002-7389-9569

REFERENCES

1. WHO. Epilepsy factsheet. 2019.
2. Ngugi AK, Bottomley C, Kleinschmidt I, Sander JW, Newton CR. Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. Epilepsia. 2010;51(5):883–90.
3. Mello LEAM, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, et al. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. Epilepsia. 1993;34(6):985–95.
4. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. Nat Rev Neurol. 2011;7(1):31–40.
5. Devinsky O, Vezzani A, Najjar S, de Lanerolle NC, Rogawski MA. Glia and epilepsy: excitability and inflammation. Trends Neurosci [Internet]. 2013;36(3):174–84. https://doi.org/10.1016/j.tins.2012.11.008
6. Sutula T, Xiao-Xian H, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. Science. 1988;239(4844):1147–50.
7. Symonds JD, Elliott KS, Shetty J, Armstrong M, Brunklaus A, Cutcutache I, et al. Early childhood epilepsies: epidemiology, classification, aetiology, and socio-economic determinants. Brain. 2021;144(9):2879–91.
8. Brennan GP, Henshall DC. MicroRNAs as regulators of brain function and targets for treatment of epilepsy. Nat Rev Neurol [Internet]. 2020;16(9):506–19.
9. Venø MT, Reschke CR, Morris G, Connolly NMC, Su J, Yan Y, et al. A systems approach delivers a functional microRNA catalog and expanded targets for seizure suppression in temporal lobe epilepsy. Proc Natl Acad Sci USA. 2020;117(27):15977–88.
10. Mula M, Kanner AM, Jetté N, Sander JW. Psychiatric comorbidities in people with epilepsy. Neurol Clin Pract [Internet]. 2021;11(2):e112–20.
11. Löscher W. Single-target versus multi-target drugs versus combinations of drugs with multiple targets: preclinical and clinical evidence for the treatment or prevention of epilepsy. Front Pharmacol. 2021;12:1–22.
12. Chen Z, Brodie MJ, Liew D, Kwan P. Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs a 30-year longitudinal cohort study. JAMA Neurol. 2018;75(3):279–86.
13. Janmohamed M, Brodie MJ, Kwan P. Pharmacoresistance – epidemiology, mechanisms, and impact on epilepsy treatment. Neuropharmacology. 2020;168:107790.

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14. Archie SR, Al SA, Cucullo L. Blood-brain barrier dysfunction in CNS disorders and putative therapeutic targets: an overview. Pharmaceutics. 2021;13(11): 1779.
15. Oby E, Janigro D. The blood-brain barrier and epilepsy. Epilepsia. 2006;47(11):1761–74.
16. Lidster K, Jefferys JG, Blümcke I, Crunelli V, Flecknell P, Frenguelli BG, et al. Opportunities for improving animal welfare in rodent models of epilepsy and seizures. J Neurosci Methods [Internet]. 2016;260:2–25.
17. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLoS Biol [Internet]. 2020;18(7):e3000410. https://doi.org/10.1371/journal.pbio.3000410
18. Raimondo JV, Heinemann U, de Curtis M, Goodkin HP, Dulla CG, Janigro D, et al. Methodological standards for in vitro models of epilepsy and epileptic seizures. A TASK1-WG4 report of the AES/ILAE Translational TASK Force of the ILAE. Epilepsia. 2017;58:40–52.
19. Dulla CG, Janigro D, Jiruska P, Raimondo JV, Ikeda A, Lin C-CK, et al. How do we use in vitro models to understand epileptiform and ictal activity? A report of the TASK1-WG4 group of the ILAE/AES Joint Translational TASK Force. Epilepsia Open. 2018;3(4):460–73.
20. Bernard C. Hippocampal slices: designing and interpreting studies in epilepsy research. Models of seizures and epilepsy. London: Elsevier Inc.; 2006. p. 59–72.
21. Colin-Le Brun I, Ferrand N, Caillard O, Tosetti P, Ben-Ari Y, Gaïarsa J-L. Spontaneous synaptic activity is required for the formation of functional GABAergic synapses in the developing rat hippocampus. J Physiol. 2004;559(Pt 1):129–39.
22. Khalilov I, Holmes GL, Ben-Ari Y. In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. Nat Neurosci. 2003;6(10):1079–85.
23. Empson RM, Heinemann U. The perforant path projection to hippocampal area CA1 in the rat hippocampal-entorhinal cortex combined slice. J Physiol. 1995;484(3):707–20.
24. von Bohlen und Halbach O, Albrecht D. Reciprocal connections of the hippocampal area CA1, the lateral nucleus of the amygdala and cortical areas in a combined horizontal slice preparation. Neurosci Res. 2002;44(1):91–100.
25. Barbarosie M, Avoli M. CA3-driven hippocampal-entorhinal loop controls rather than sustains in vitro limbic seizures. J Neurosci. 1997;17(23):9308–14.
26. D'Antuono M, Benini R, Biagini G, D'Arcangelo G, Barbarosie M, Tancredi V, et al. Limbic network interactions leading to hyperexcitability in a model of temporal lobe epilepsy. J Neurophysiol. 2002;87(1):634–9.
27. Borck C, Jefferys JG. Seizure-like events in disinhibited ventral slices of adult rat hippocampus. J Neurophysiol. 1999;82(5):2130–42.
28. Traynelis SF, Dingledine R. Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. J Neurophysiol [Internet]. 1988;59:259–76.
29. Morris G, Jiruska P, Jefferys JGR, Powell AD. A new approach of modified submerged patch clamp recording reveals interneuronal dynamics during epileptiform oscillations. Front Neurosci. 2016;10:519.
30. Mody I, Lambert JDC, Heinemann U. Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. J Neurophysiol. 1987;57(3):869–88.
31. Jefferys JGR, Haas HL. Synchronized bursting of CA1 hippocampal pyramidal cells in the absence of synaptic transmission. Nature. 1982;300(5891):448–50.
32. Fueta Y, Avoli M. Effects of antiepileptic drugs on 4-aminopyridine-induced epileptiform activity in young and adult rat hippocampus. Epilepsy Res. 1992;12(3):207–15.
33. Straub H, Köhling R, Speckmann EJ. Picrotoxin-induced epileptic activity in hippocampal and neocortical slices (Guinea pig): suppression by organic calcium channel blockers. Brain Res. 1994;658(1–2):119–26.
34. Khalilov I, Khazipov R, Esclapez M, Ben-Ari Y. Bicuculline induces ictal seizures in the intact hippocampus recorded in vitro. Eur J Pharmacol. 1997;319(2–3):5–6.
35. Bingmann D, Speckmann E-J. Actions of pentylenetetrazol (PTZ) on CA3 neurons in hippocampal slices of Guinea pigs. Exp Brain Res. 1986;64(1):94–104.
36. Fujiwara-Tsukamoto Y, Isomura Y, Nambu A, Takada M. Excitatory gaba input directly drives seizure-like rhythmic synchronization in mature hippocampal CA1 pyramidal cells. Neuroscience. 2003;119(1):265–75.
37. Ridler T, Matthews P, Phillips KG, Randall AD, Brown JT. Initiation and slow propagation of epileptiform activity from ventral to dorsal medial entorhinal cortex is constrained by an inhibitory gradient. J Physiol. 2018;596(11):2251–66.
38. Luhmann HJ, Dzhala VI, Ben-Ari Y. Generation and propagation of 4-AP-induced epileptiform activity in neonatal intact limbic structures in vitro. Eur J Neurosci. 2000;12(8):2757–68.
39. Mikroulis A, Psarropoulou C. Endogenous ACh effects on NMDA-induced interictal-like discharges along the septotemporal hippocampal axis of adult rats and their modulation by an early life generalized seizure. Epilepsia. 2012;53(5): 879–87.
40. Chan F, Lax NZ, Voss CM, Aldana BI, Whyte S, Jenkins A, et al. The role of astrocytes in seizure generation: insights from a novel in vitro seizure model based on mitochondrial dysfunction. Brain. 2019;142(2):391–411.
41. Nikkanen J, Forsström S, Euro L, Paetau I, Kohnz RA, Wang L, et al. Mitochondrial DNA replication defects disturb cellular dNTP pools and remodel one-carbon metabolism. Cell Metab. 2016;23(4):635–48.
42. Jirsa VK, Stacey WC, Quilichini PP, Ivanov AI, Bernard C. On the nature of seizure dynamics. Brain [Internet]. 2014;137(8):2210–30.
43. Graham RT, Parrish RR, Alberio L, Johnson EL, Owens L, Trevelyan AJ. Optogenetic stimulation reveals a latent tipping point in cortical networks during ictogenesis. Brain. 2022;146(7):2814–27.
44. Le Duigou C, Bouilleret V, Miles R. Epileptiform activities in slices of hippocampus from mice after intra-hippocampal injection of kainic acid. J Physiol. 2008;586(20):4891–904.
45. West PJ, Saunders GW, Billingsley P, Smith MD, White HS, Metcalf CS, et al. Recurrent epileptiform discharges in the medial entorhinal cortex of kainate-treated rats are differentially sensitive to antiseizure drugs. Epilepsia. 2018;59(11):2035–48.
46. Jefferys JG. Chronic epileptic foci in vitro in hippocampal slices from rats with the tetanus toxin epileptic syndrome. J Neurophysiol. 1989;62(2):458–68.
47. Stein RE, Kaplan JS, Li J, Catterall WA. Hippocampal deletion of NaV1.1 channels in mice causes thermal seizures and cognitive deficit characteristic of Dravet syndrome. Proc Natl Acad Sci USA. 2019;116(33):16571–76.

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

48. Jones RSG, da Silva AB, Whittaker RG, Woodhall GL, Cunningham MO. Human brain slices for epilepsy research: pitfalls, solutions and future challenges. J Neurosci Methods [Internet]. 2016;260:221–32. https://doi.org/10.1016/j.jneumeth.2015.09.021
49. Gabriel S, Njunting M, Pomper JK, Merschhemke M, Sanabria ERG, Eilers A, et al. Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. J Neurosci. 2004;24(46):10416–30.
50. Huberfeld G, Blauwblomme T, Miles R. Hippocampus and epilepsy: findings from human tissues. Rev Neurol (Paris). 2015;171(3):236–51.
51. Toyoda I, Bower MR, Leyva F, Buckmaster PS. Early activation of ventral hippocampus and subiculum during spontaneous seizures in a rat model of temporal lobe epilepsy. J Neurosci. 2013;33(27):11100–15.
52. Sheybani L, van Mierlo P, Birot G, Michel CM, Quairiaux C. Large-scale 3–5 Hz oscillation constrains the expression of neocortical fast ripples in a mouse model of mesial temporal lobe epilepsy. eNeuro. 2019;6(1):ENEURO.0494-18.2019.
53. Khalilov I, Esclapez M, Medina I, Aggoun D, Lamsa K, Leinekugel X, et al. A novel in vitro preparation: the intact hippocampal formation. Neuron. 1997;19:743–9.
54. Quilichini PP, Diabira D, Chiron C, Ben-Ari Y, Gozlan H. Persistent epileptiform activity induced by low Mg2+ in intact immature brain structures. Eur J Neurosci. 2002;16(5):850–60.
55. Quilichini PP, Diabira D, Chiron C, Milh M, Ben-Ari Y, Gozlan H. Effects of antiepileptic drugs on refractory seizures in the intact immature corticohippocampal formation in vitro. Epilepsia. 2003;44(11):1365–74.
56. Mühlethaler M, de Curtis M, Walton K, Llinás R. The isolated and perfused brain of the guinea-pig in vitro. Eur J Neurosci. 1993;5(7):915–26.
57. Hájos N, Ellender TJ, Zemankovics R, Mann EO, Exley R, Cragg SJ, et al. Maintaining network activity in submerged hippocampal slices: importance of oxygen supply. Eur J Neurosci. 2009;29(2):319–27.
58. de Curtis M, Takashima I, Iijima T. Optical recording of cortical activity after in vitro perfusion of cerebral arteries with a voltage-sensitive dye. Brain Res. 1999;837(1–2):314–9.
59. Biella G, Spaiardi P, Toselli M, de Curtis M, Gnatkovsky V. Functional interactions within the parahippocampal region revealed by voltage-sensitive dye imaging in the isolated guinea pig brain. J Neurophysiol. 2010;103(2):725–32.
60. Federico P, Borg SG, Salkauskus AG, MacVicar BA. Mapping patterns of neuronal activity and seizure propagation by imaging intrinsic optical signals in the isolated whole brain of the guinea-pig. Neuroscience. 1994;58(3):461–80.
61. Gómez-Gonzalo M, Losi G, Chiavegato A, Zonta M, Cammarota M, Brondi M, et al. An excitatory loop with astrocytes contributes to drive neurons to seizure threshold. PLoS Biol. 2010;8(4):e1000352.
62. de Curtis M, Librizzi L, Uva L, Gnatkovsky V. Neuronal networks in the in vitro isolated guinea pig brain. Totowa, NJ: Humana Press; 2012. p. 357–83.
63. Mazzetti S, Librizzi L, Frigerio S, de Curtis M, Vitellaro-Zuccarello L. Molecular anatomy of the cerebral microvessels in the isolated Guinea-pig brain. Brain Res. 2004;999(1):81–90.
64. Librizzi L, Janigro D, de Biasi S, de Curtis M. Blood-brain barrier preservation in the in vitro isolated Guinea pig brain preparation. J Neurosci Res. 2001;66(2):289–97.
65. van Vliet EA, Marchi N. Neurovascular unit dysfunction as a mechanism of seizures and epilepsy during aging. Epilepsia. 2022;63(6):1297–313.
66. Uva L, Librizzi L, Marchi N, Noe F, Bongiovanni R, Vezzani A, et al. Acute induction of epileptiform discharges by pilocarpine in the in vitro isolated guinea-pig brain requires enhancement of blood–brain barrier permeability. Neuroscience. 2008;151(1):303–12.
67. Librizzi L, Vila Verde D, Colciaghi F, Deleo F, Regondi MC, Costanza M, et al. Peripheral blood mononuclear cell activation sustains seizure activity. Epilepsia. 2021;62(7):1715–28.
68. Librizzi L, Noè F, Vezzani A, de Curtis M, Ravizza T. Seizure-induced brain-borne inflammation sustains seizure recurrence and blood-brain barrier damage. Ann Neurol. 2012;72(1):82–90.
69. Librizzi L, de Cutis M, Janigro D, Runtz L, de Bock F, Barbier EL, et al. Cerebrovascular heterogeneity and neuronal excitability. Neurosci Lett. 2018;667:75–83.
70. Librizzi L, Pastori C, de Grazia U, Croci D, de Curtis M. Rapid in vitro elimination of anesthetic doses of thiopental in the isolated Guinea pig brain. Neurosci Lett. 2005;380(1–2):66–9.
71. Gähwiler BH, Capogna M, Debanne D, McKinney RA, Thompson SM. Organotypic slice cultures: a technique has come of age. Trends Neurosci. 1997;20(10):471–7.
72. Stoppini L, Buchs PA, Muller D. A simple method for organotypic cultures of nervous tissue. J Neurosci Methods. 1991;37(2):173–82.
73. Humpel C. Neuroscience forefront review organotypic brain slice cultures: a review. Neuroscience. 2015;305:86–98.
74. Morris G, O'Brien D, Henshall DC. Opportunities and challenges for microRNA-targeting therapeutics for epilepsy. Trends Pharmacol Sci [Internet]. 2021;42(7):605–16. https://doi.org/10.1016/j.tips.2021.04.007
75. Snowball A, Chabrol E, Wykes RC, Shekh-Ahmad T, Cornford JH, Lieb A, et al. Epilepsy gene therapy using an engineered potassium channel. J Neurosci. 2019;39(16):3159–69.
76. Kullmann DM, Schorge S, Walker MC, Wykes RC. Gene therapy in epilepsy—is it time for clinical trials? Nat Rev Neurol [Internet]. 2014;10(5):300–4.
77. McBain CJ, Boden P, Hill RG. Rat hippocampal slices "in vitro" display spontaneous epileptiform activity following long-term organotypic culture. J Neurosci Methods. 1989;27(1):35–49.
78. Lau LA, Staley KJ, Lillis KP. In vitro ictogenesis is stochastic at the single neuron level. Brain. 2022;145(2):531–41.
79. Berdichevsky Y, Saponjian Y, Park K, Roach B, Pouliot W, Lu K, et al. Staged anticonvulsant screening for chronic epilepsy. Ann Clin Transl Neurol. 2016;3(12):908–23.
80. Obergrussberger A, Rinke-Weiß I, Goetze TA, Rapedius M, Brinkwirth N, Becker N, et al. The suitability of high throughput automated patch clamp for physiological applications. J Physiol. 2022;600(2):277–97.
81. Vanoye CG, Desai RR, Ji Z, Adus S, Jairam N, Joshi N, et al. High-throughput evaluation of epilepsy-associated KCNQ2 variants reveals functional and pharmacological heterogeneity. JCI Insight. 2021;7:e156314.

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

82. Barilli A, Aldegheri L, Bianchi F, Brault L, Brodbeck D, Castelletti L, et al. From high-throughput screening to target validation: benzo[d]isothiazoles as potent and selective agonists of human transient receptor potential cation channel subfamily M member 5 possessing in vivo gastrointestinal prokinetic activity in rodents. J Med Chem. 2021;64(9):5931–55.
83. Bar-Yehuda D, Korngreen A. Space-clamp problems when voltage clamping neurons expressing voltage-gated conductances. J Neurophysiol. 2008;99(3):1127–36.
84. Neher E, Sakmann B. Single-channel currents recorded from membrane. Nature [Internet]. 1976;260:799–802.
85. Marguet SL, Le-Schulte VTQ, Merseburg A, Neu A, Eichler R, Jakovcevski I, et al. Treatment during a vulnerable developmental period rescues a genetic epilepsy. Nat Med. 2015;21(12):1436–44.
86. Marder E, Taylor AL. Multiple models to capture the variability in biological neurons and networks. Nat Neurosci. 2011;14(2):133–8.
87. Saneto RP. Preparation of highly purified populations of neurons, astrocytes, and oligodendrocytes. Methods Neurosci. 1990;2:119–33.
88. Furshpan EJ, Potter DD. Seizure-like activity and cellular damage in rat hippocampal neurons in cell culture. Neuron. 1989;3(2):199–207.
89. Segal MM. Epileptiform activity in microcultures containing one excitatory hippocampal neuron. J Neurophysiol. 1991;65(4):761–70.
90. Wei Y, Ullah G, Schiff SJ. Unification of neuronal spikes, seizures, and spreading depression. J Neurosci. 2014;34(35):11733–43.
91. Gonzalez-Sulser A, Wang J, Motamedi GK, Avoli M, Vicini S, Dzakpasu R. The 4-aminopyridine in vitro epilepsy model analyzed with a perforated multi-electrode array. Neuropharmacology. 2011;60(7–8):1142–53.
92. Gonzalez-Sulser A, Wang J, Queenan BN, Avoli M, Vicini S, Dzakpasu R. Hippocampal neuron firing and local field potentials in the in vitro 4-aminopyridine epilepsy model. J Neurophysiol. 2012;108(9):2568–80.
93. Morris G, Leite M, Kullmann D, Pavlov I, Schorge S, Lignani G, et al. Activity clamp provides insights into paradoxical effects of the anti-seizure drug carbamazepine. J Neurosci [Internet]. 2017;37(22):5484–95.
94. Kovac S, Domijan A-M, Walker MC, Abramov AY. Seizure activity results in calcium- and mitochondria-independent ROS production via NADPH and xanthine oxidase activation. Cell Death Dis. 2014;5(10):e1442.
95. Kovac S, Domijan A-M, Walker MC, Abramov AY. Prolonged seizure activity impairs mitochondrial bioenergetics and induces cell death. J Cell Sci. 2012;125:1796–806.
96. Malik N, Rao MS. A review of the methods for human iPSC derivation. Methods Mol Biol. 2013;997(5):23–33.
97. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663–76.
98. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861–72.
99. Shi Y, Inoue H, Wu JC, Yamanaka S. Induced pluripotent stem cell technology: a decade of progress. Nat Rev Drug Discov. 2017;16(2):115–30.
100. Lancaster MA, Corsini NS, Wolfinger S, Gustafson EH, Phillips AW, Burkard TR, et al. Guided self-organization and cortical plate formation in human brain organoids. Nat Biotechnol. 2017;35(7):659–66.
101. Fair SR, Julian D, Hartlaub AM, Pusuluri ST, Malik G, Summerfied TL, et al. Electrophysiological maturation of cerebral organoids correlates with dynamic morphological and cellular development. Stem Cell Reports. 2020;15(4):855–68.
102. Samarasinghe RA, Miranda OA, Buth JE, Mitchell S, Ferando I, Watanabe M, et al. Identification of neural oscillations and epileptiform changes in human brain organoids. Nat Neurosci. 2021;24(10):1488–500.
103. Steinberg DJ, Repudi S, Saleem A, Kustanovich I, Viukov S, Abudiab B, et al. Modeling genetic epileptic encephalopathies using brain organoids. EMBO Mol Med. 2021;13(8):e13610.
104. Qiu Y, O'Neill N, Maffei B, Zourray C, Almacellas-Barbanoj A, Carpenter JC, et al. On-demand cell-autonomous gene therapy for brain circuit disorders. Science. 2022;378(6619):523–32.
105. Morris G, Rowell R, Cunningham MO. Limitations of animal epilepsy research models: can epileptic human tissue provide translational benefit? ALTEX [Internet]. 2021;38:451–62.
106. Jobst BC, Cascino GD. Resective epilepsy surgery for drug-resistant focal epilepsy: a review. JAMA. 2015;313(3):285–93.
107. Wickham J, Brödjegård NG, Vighagen R, Pinborg LH, Bengzon J, Woldbye DPD, et al. Prolonged life of human acute hippocampal slices from temporal lobe epilepsy surgery. Sci Rep. 2018;8(1):1–13.
108. Morris G, Langa E, Fearon C, Conboy K, Lau E-How K, Sanz-Rodriguez A, et al. MicroRNA inhibition using antimiRs in acute human brain tissue sections. Epilepsia. 2022;63:e92–9.
109. Schwarz N, Uysal B, Welzer M, Bahr JC, Layer N, Löffler H, et al. Long-term adult human brain slice cultures as a model system to study human CNS circuitry and disease. Elife. 2019;8:1–26.
110. Schwarz N, Hedrich UBS, Schwarz H, Harshad PA, Dammeier N, Auffenberg E, et al. Human cerebrospinal fluid promotes long-term neuronal viability and network function in human neocortical organotypic brain slice cultures. Sci Rep. 2017;7(1):1–12.
111. McLeod F, Dimtsi A, Marshall AC, Lewis-Smith D, Thomas R, Clowry GJ, et al. Altered synaptic connectivity in an in vitro human model of STXBP1 encephalopathy. Brain. 2022;146:850–7.
112. Kovács R, Raue C, Gabriel S, Heinemann U. Functional test of multidrug transporter activity in hippocampal–neocortical brain slices from epileptic patients. J Neurosci Methods. 2011;200(2):164–72.
113. Roopun AK, Simonotto JD, Pierce ML, Jenkins A, Nicholson C, Schofield IS, et al. A nonsynaptic mechanism underlying interictal discharges in human epileptic neocortex. Proc Natl Acad Sci USA. 2010;107(1):338–43.
114. Pallud J, le Van Quyen M, Bielle F, Pellegrino C, Varlet P, Labussiere M, et al. Cortical GABAergic excitation contributes to epileptic activities around human glioma. Sci Transl Med. 2014;6(244):244ra89.
115. Huberfeld G, Menendez de la Prida L, Pallud J, Cohen I, le Van Quyen M, Adam C, et al. Glutamatergic pre-ictal discharges emerge at the transition to seizure in human epilepsy. Nat Neurosci. 2011;14(5):627–34.

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

116. Schwartzkroin PA, Haglund MM. Spontaneous rhythmic synchronous activity in epileptic human and Normal monkey temporal lobe. Epilepsia. 1986;27(5):523–33.
117. Köhling R, Lücke A, Straub H, Speckmann EJ, Tuxhorn I, Wolf P, et al. Spontaneous sharp waves in human neocortical slices excised from epileptic patients. Brain. 1998;121(Pt 6):1073–87.
118. Taing KD, O'Brien TJ, Williams DA, French CR. Anti-epileptic drug combination efficacy in an in vitro seizure model – phenytoin and valproate, lamotrigine and valproate. PLoS One. 2017;12(1):e0169974.
119. Whittington MA, Traub RD, Jefferys JG. Erosion of inhibition contributes to the progression of low magnesium bursts in rat hippocampal slices. J Physiol. 1995;486(3):723–34.
120. Dreier JP, Zhang C-L, Heinemann U. Phenytoin, phenobarbital, and midazolam fail to stop status epilepticus-like activity induced by low magnesium in rat entorhinal slices, but can prevent its development. Acta Neurol Scand. 1998;98(3):154–60.
121. Burman RJ, Selfe JS, Lee JH, van den Berg M, Calin A, Codadu NK, et al. Excitatory GABAergic signalling is associated with benzodiazepine resistance in status epilepticus. Brain. 2019;142(11):3482–501.
122. D'Antuono M, Köhling R, Ricalzone S, Gotman J, Biagini G, Avoli M. Antiepileptic drugs abolish ictal but not interictal epileptiform discharges in vitro. Epilepsia. 2010;51(3):423–31.
123. Avoli M, de Curtis M. GABAergic synchronization in the limbic system and its role in the generation of epileptiform activity. Prog Neurobiol. 2011;95(2):104–32.
124. Heuzeroth H, Wawra M, Fidzinski P, Dag R, Holtkamp M. The 4-aminopyridine model of acute seizures in vitro elucidates efficacy of new antiepileptic drugs. Front Neurosci. 2019;13:677.
125. Morris G, Heiland M, Lamottke K, Guan H, Hill TDM, Zhou Y, et al. BICS01 mediates reversible anti-seizure effects in brain slice models of epilepsy. Front Neurol. 2022;12:791608.
126. Hall BJ, Satterfield-Doerr M, Parikh AR, Brodbelt JS. Determination of cannabinoids in water and human saliva by solid-phase microextraction and quadrupole ion trap gas chromatography/mass spectrometry. Anal Chem. 1998;70(9):1788–96.
127. Mantegazza M, Curia G, Biagini G, Ragsdale DS, Avoli M. Voltage-gated sodium channels as therapeutic targets in epilepsy and other neurological disorders. Lancet Neurol. 2010;9:413–24.
128. Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, et al. Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. J Neurosci. 2007;27(22):5903–14.
129. Gidon A, Zolnik TA, Fidzinski P, Bolduan F, Papoutsi A, Poirazi P, et al. Dendritic action potentials and computation in human layer 2/3 cortical neurons. Science. 2020;367(6473):83–7.
130. Boldog E, Bakken TE, Hodge RD, Novotny M, Aevermann BD, Baka J, et al. Transcriptomic and morphophysiological evidence for a specialized human cortical GABAergic cell type. Nat Neurosci. 2018;21(9):1185–95.
131. Wickham J, Corna A, Schwarz N, Uysal B, Layer N, Honegger JB, et al. Human cerebrospinal fluid induces neuronal excitability changes in resected human neocortical and hippocampal brain slices. Front Neurosci. 2020;14:1–14.
132. Deisseroth K, Feng G, Majewska AK, Miesenböck G, Ting A, Schnitzer MJ. Next-generation optical technologies for illuminating genetically targeted brain circuits. J Neurosci. 2006;26(41):10380–86.
133. Panzera LC, Hoppa MB. Genetically encoded voltage indicators are illuminating subcellular physiology of the axon. Front Cell Neurosci. 2019;13:1–9.
134. Emiliani V, Cohen AE, Deisseroth K, Häusser M. All-optical interrogation of neural circuits. J Neurosci. 2015;35(41):13917–26.
135. Nakai J, Ohkura M, Imoto K. A high signal-to-noise Ca(2+) probe composed of a single green fluorescent protein. Nat Biotechnol. 2001;19(2):137–41.
136. Marvin JS, Borghuis BG, Tian L, Cichon J, Harnett MT, Akerboom J, et al. An optimized fluorescent probe for visualizing glutamate neurotransmission. Nat Methods. 2013;10(2):162–70.
137. Marvin JS, Shimoda Y, Magloire V, Leite M, Kawashima T, Jensen TP, et al. A genetically encoded fluorescent sensor for in vivo imaging of GABA. Nat Methods. 2019;16(8):763–70.
138. Zaaimi B, Turnbull M, Hazra A, Wang Y, Gandara C, McLeod F, et al. Closed-loop optogenetic control of the dynamics of neural activity in non-human primates. Nat Biomed Eng. 2022;7(4):559–75.
139. Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature. 2009;459(7247):698–702.
140. Morris G, Schorge S. Gene therapy for neurological disease: state of the art and opportunities for next-generation approaches. Neuroscience. 2022;490:309–14.

How to cite this article: Morris G, Avoli M, Bernard C, Connor K, de Curtis M, Dulla CG, et al. Can in vitro studies aid in the development and use of antiseizure therapies? A report of the ILAE/AES Joint Translational Task Force. Epilepsia. 2023;00:1–15. https://doi.org/10.1111/epi.17744

15281167, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.17744 by University College London UCL Library Services, Wiley Online Library on [04/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License