Crosstalk opposing view: Animal models of epilepsy are more useful than human tissue-based approaches

Running title: Animal models are more useful than human models of epilepsy

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Epilepsy and models

Epilepsy is one of the most common severe neurological diseases, affecting up to 70 million people worldwide (Ngugi *et al.*, 2010). It is characterised by an imbalance of neuronal network excitation and inhibition which causes spontaneous recurrent seizures, as well as cognitive and psychiatric co-morbidities (Mula *et al.*, 2021). The underlying pathophysiology varies between individuals and epilepsy types. It can be complex and multi-faceted, including genetic (Carpenter & Lignani, 2021) and transcriptomic (Venø *et al.*, 2020) dysregulations, cell loss (Mello *et al.*, 1993), gliosis (Devinsky *et al.*, 2013), neuroinflammation (Vezzani *et al.*, 2011) and circuit dysfunction (Goldberg & Coulter, 2013). Valid disease models are critical to basic understanding and developing new therapies for the epilepsies. Many models are available, ranging from *in silico* simulations (Liou *et al.*, 2020) to *ex vivo* brain slice models (Morris *et al.*, 2016) and *in vivo* animal studies (Kandratavicius *et al.*, 2014; Wang *et al.*, 2022). Multiple species are amenable to these models, with each approach having advantages and disadvantages. The nature of certain epilepsies provides an opportunity for study using human brain slice models (Morris *et al.*, 2021*b*), because surgical resection of the seizure focus remains the best therapeutic pathway for certain patients (Jette *et al.*, 2014). Human tissue-based models can also include induced pluripotent stem cell (iPSC) cultures from epilepsy patients, or three-dimensional organoids grown from these cells (Shi *et al.*, 2017). Models can be assessed in terms of three types of validity (van der Staay, 2006): 1) face validity – the ability to capture relevant disease phenotypes; 2) construct validity – the disease model is caused by the same precipitating insult as the real disease; 3) predictive validity – the model predicts the response of the real disease to interventions. Here, we argue that, despite advances in human tissue models, animal models of epilepsy remain more useful for interrogating basic mechanisms and novel therapeutics in epilepsy.

Modelling different epilepsies and epileptogenesis

A key advantage of animal models is the ability to reproducibly capture epilepsies with different aetiologies. In rodents, it is possible to elicit acute seizures, status epilepticus and/or chronic epilepsy using a variety of means (Kandratavicius *et al.*, 2014; Wang *et al.*, 2022). Rodent acquired epilepsy models include chemoconvulsants which typically increase excitation (e.g. (Mouri *et al.*, 2008)) or reduce inhibition (Jiruska et al., 2010), electrical

stimulation (Norwood *et al.*, 2010) and traumatic brain injury (Santana-Gomez et al., 2021). Some species - particularly mice and zebrafish (Grone & Baraban, 2015) - are highly amenable to genetic manipulations, offering realistic whole brain models of developmental epileptic encephalopathies s (DEEs, e.g. Dravet syndrome (Ogiwara *et al.*, 2007)). Moreover, because animal models can be tracked from initial precipitating insult right through to chronic epilepsy (Venø *et al.*, 2020), they also provide a tool to study mechanisms of epileptogenesis, which is not feasible using resected human tissue that has already undergone epileptogenesis. All of the listed models have good face validity, generating seizures which are the main hallmark of epilepsy. The variety of mechanisms to induce epilepsy means that construct validity is often strong, and this is particularly the case for those genetic epilepsies where real human mutations can be modelled in animals. Many of these models are also often treatment resistant, supporting predictive validity in terms of modelling drug-resistant epilepsies. The availability of many types of animal model allows researchers to interrogate pathophysiology and novel therapeutics for efficacy in different types of seizures, therefore avoiding model-specific observations (e.g. (Venø et al., 2020)).

In contrast, resected human tissue slices can only be obtained from a specific subset of patients. Surgical resection is only performed in drug-resistant focal epilepsy, when a person's seizures are not completely resolved by anti-seizure drugs (ASDs). This represents a minority of patients (Löscher *et al.*, 2020); moreover surgical resection is not indicated in all such patients, for example if the epileptogenic zone overlaps with eloquent cortex (Jette *et al.*, 2014). Therefore, resected human tissue models represent a narrow subset of epilepsies, and do not support investigation of other disease types. Notably, DEEs are typically severe and drug-resistant childhood epilepsies with limited treatment options (Carpenter & Lignani, 2021). DEEs are often caused by *de novo* mutations in key epilepsy-related genes, and their pathophysiology may be entirely different to resected tissue sections which are typically only obtained from focal epilepsy patients. This lack of genetic epilepsy tissue is partially balanced by iPSCs and organoids derived from DEE patients (Shi *et al.*, 2017), but whether these form realistic brain networks is debatable. Therefore, a significant advantage of animal models is that they accurately capture a wide-range of disease aetiologies.

Whole brain studies and peripheral effects

Another fundamental advantage of animal models is the whole brain nature of these approaches. Whilst the rodent brain might not capture the full scale of the human brain, it is a comparable representation (Semple *et al.*, 2013) and allows interrogation of epileptic pathophysiology at the level of the whole brain, rather than within specific microcircuits. Indeed, epilepsy is a disease of brain networks, therefore it is important that epilepsy models retain widespread anatomical connectivity. Extending this idea, whole animal *in vivo* studies permit assessment of the peripheral effects of systemic interventions. This is an essential part of preclinical safety testing and is not possible in humans prior to formal clinical trials. In the case of human tissue models, specimens are disconnected from their wider anatomical projections and so it is not possible to model parameters such as seizure propagation or the interaction between distant interconnected anatomical structures.

Co-morbidities and non-seizure aspects of epilepsy

Whilst epilepsy has classically been most closely associated with seizures, it is increasingly recognised that co-morbidities can include challenges with memory, sleep and mental health (Mula *et al.*, 2021). Indeed, people with epilepsy and their carers report that co-morbidities often have a greater impact on their daily lives than seizures do. Animal models allow investigation of disease pathophysiology and novel anti-epileptic medications in the context of these 'non-seizure' aspects of epilepsy. Wide-ranging behavioural paradigms are available to probe specific facets of memory in rodents (Hattiangady *et al.*, 2014). Long-term video-EEG telemetry can interrogate the effects of interventions on sleep. It is admittedly not clear how accurately human psychiatric co-morbidities can be captured in animal models, but behavioural testing can be used to explore basic phenotypes such as anxiety (Harro, 2018). Whilst human tissue models may be able to capture epileptiform activity, it is challenging to extrapolate this activity to impacts on other brain functions.

Experimental logistics and reproducibility

Animal-based experiments offer significant advantages in experimental design, reproducibility and predictability. One such advantage is straightforward access to relevant healthy control tissue by using non-epileptic animals. This is not possible with human slices where the tissue is diseased, precluding certain types of study because healthy human brain samples are not available as controls. A second consideration is the variability of samples. In

animal studies, experimental models can be designed to be relatively homogeneous, with standardised and reproducible procedures to model epilepsy in animals from identical genetic backgrounds. This allows the researcher to easily isolate experimental variables of interest, with good control over confounding variables that may influence experimental readouts such as age, sex, husbandry, and time of day when tissue is prepared. This is not the case with human tissues, where these factors cannot be controlled. Moreover chronic drug treatment in patients, prior to tissue resection, is likely to lead to altered neuronal physiology and potentially affects treatment outcomes when trialling new therapeutics. Human tissue samples are therefore inherently more heterogeneous, necessitating greater sample sizes for robust statistical comparisons. Human samples are less regularly available than mouse models, since they are obtained from relatively infrequent surgical procedures. The need for larger sample sizes, coupled with relatively limited availability of tissues, mean that human-based models have much lower throughput than studies in animals.

Techniques which can be used in animal models

In addition to these advantages in scientific reproducibility, animal models are also amenable to a range of experimental techniques that can be applied to study epilepsy, most notably *in vivo* experiments and expression of exogenous genetic products. The ability to introduce exogenous genetic material into animal models has sparked advances in optogenetics (Deisseroth *et al.*, 2006; Magloire *et al.*, 2019)and chemogenetics (Lieb *et al.*, 2019) to study epileptic networks, in addition to antisense oligonucleotide (Morris *et al.*, 2021*a*) and gene therapies as novel treatment options (Kullmann *et al.*, 2014; Morris & Schorge, 2022).

Viral vectors are often used to express exogenous proteins in the intact brain for both *in vivo* and *in vitro* purposes, such as calcium imaging or electrophysiology. Robust expression of genes contained in viral vectors can take several weeks and as such, the transduced tissue needs to be viable for long periods of time. This introduces challenges for using these techniques within *in vitro* preparations of human brain tissue. Recent progress in culturing human tissue slices allows introduction of viral vectors *in vitro* (Schwarz *et al.*, 2017), but the limited section of tissue occludes the ability to probe larger networks as can be performed using animal models, and organotypic slices spontaneously change their circuit physiology over time. Our understanding of large-scale epileptic networks has been

advanced in recent years using optogenetics to probe interactions between interconnected brain regions during epileptic activity (e.g. (Streng & Krook-Magnuson, 2019)), in addition to revealing interactions between inhibitory and excitatory neuronal subpopulations on the microcircuit level (Ellender et al., 2014). Similarly *in vivo* electrophysiology and imaging in rodent and primate models has vastly increased understanding of cellular recruitment during seizures (Wenzel *et al.*, 2017) in intact circuits, identifying new targets for epilepsy treatments. In addition, use of depth electrodes and EEG recording devices *in vivo* allows for examining efficacy of novel therapeutics during chronic epilepsy studies all of which is currently impossible using available human models.

Animal models have been vital to recent advances in novel therapeutics- most notably the development of gene therapies and antisense oligonucleotides as long-acting treatments for epilepsy (Kullmann *et al.*, 2014; Morris & Schorge, 2022). Typically these treatments would be injected intracranially or intrathecally and would be active for periods of months to years. Examining the efficacy of these therapeutics requires long-term chronic studies. Not only do the genetic manipulations required to develop these therapeutics require robust animal models but chronic studies needed to determine safety and efficacy are not currently possible with human tissue models. Thus, animal models are crucial for the development of novel advanced treatment options for epilepsy patients.

Ethical considerations

Both animal models and the use of human brain tissues raise ethical issues which should be considered when designing research studies. In the case of animal models, studies typically require local approval by an institutional animal welfare body, and must be performed by trained and licenced personnel working under an approved project licence. These safeguards ensure that any animal work performed is necessary and ethical. In line with the 3Rs, animal use should be reduced, refined and replaced wherever possible. Indeed, human tissues offer a replacement for animal use, but this approach is associated with other ethical concerns. Human tissue studies must be approved by ethical boards and, in the UK, comply with the Human Tissue Act. Patients donating tissue must be able to give fully informed consent, prior to their surgical procedure. Data protection is also a concern when using human tissues. Patient data must be anonymised and held securely. There are also implications for biobanking of tissue. With animal models, there are no additional

concerns about the long-term storage of tissues and indeed re-use of banked tissue for future studies is an ideal way to reduce animal usage. For human tissues, long-term storage may represent a severe privacy concern, and specimens must not be banked or used for further studies without explicit consent.

Conclusions

The epilepsies are a range of complex and often multi-faceted conditions. As such there are numerous types of epilepsy models which capture different aspects of the disease. In choosing a model it is critical that it captures disease pathophysiology with maximal construct, face and predictive validity. It must be noted that despite the views presented, human-based models also have advantages, most notably in construct validity, over animal studies. Researchers must carefully select the type of model most appropriate to their research question and it is not necessarily the case that one type of model is inherently more useful than another for every experiment. Here, we present numerous examples of research questions which can only be answered using animal models.

Reference List

Carpenter JC & Lignani G (2021). Gene Editing and Modulation: the Holy Grail for the Genetic Epilepsies? *Neurotherapeutics*; DOI: 10.1007/s13311-021-01081-y.

Deisseroth K, Feng G, Majewska AK, Miesenböck G, Ting A & Schnitzer MJ (2006). Next-generation optical technologies for illuminating genetically targeted brain circuits. *Journal of Neuroscience* **26,** 10380–10386.

Devinsky O, Vezzani A, Najjar S, de Lanerolle NC & Rogawski MA (2013). Glia and epilepsy: Excitability and inflammation. *Trends in Neurosciences* **36,** 174–184.

Ellender TJ, Raimondo J v., Irkle A, Lamsa KP & Akerman CJ (2014). Excitatory effects of parvalbumin-expressing interneurons maintain hippocampal epileptiform activity via synchronous afterdischarges. *Journal of Neuroscience* **34,** 15208–15222.

Goldberg EM & Coulter DA (2013). Mechanisms of epileptogenesis: A convergence on neural circuit dysfunction. *Nature Reviews Neuroscience* **14,** 337–349.

Grone BP & Baraban SC (2015). Animal models in epilepsy research: Legacies and new directions. *Nature Neuroscience* **18,** 339–343.

Harro J (2018). Animals, anxiety, and anxiety disorders: How to measure anxiety in rodents and why. *Behavioural Brain Research* **352,** 81–93.

Hattiangady B, Mishra V, Kodali M, Shuai B, Rao X & Shetty AK (2014). Object location and object recognition memory impairments, motivation deficits and depression in a model of Gulf War illness. *Frontiers in Behavioral Neuroscience* **8,** 1–10.

Jette N, Reid AY & Wiebe S (2014). Surgical management of epilepsy. *Canadian Medical Association Journal* **186,** 997–1004.

Jiruska P, Finnerty GT, Powell AD, Lofti N, Cmejla R & Jefferys JGR (2010). Epileptic high-frequency network activity in a model of non-lesional temporal lobe epilepsy. *Brain* **133,** 1380–1390.

Kandratavicius L, Alves Balista P, Lopes-Aguiar C, Ruggiero RN, Umeoka EH, Garcia-Cairasco N, Soares Bueno-Junior L & Leite JP (2014). Animal models of epilepsy: use and limitations. *Neuropsychiatric Disease and Treatment*1693–1705.

Kullmann DM, Schorge S, Walker MC & Wykes RC (2014). Gene therapy in epilepsy—is it time for clinical trials? *Nature Reviews Neurology* **10,** 300–304.

Lieb A, Weston M & Kullmann DM (2019). Designer receptor technology for the treatment of epilepsy. *EBioMedicine* **43,** 641–649.

Liou J, Smith EH, Bateman LM, Bruce SL, McKhann GM, Goodman RR, Emerson RG, Schevon CA & Abbott L (2020). A model for focal seizure onset, propagation, evolution, and progression. *Elife*; DOI: 10.7554/eLife.50927.

Löscher W, Potschka H, Sisodiya SM & Vezzani A (2020). Drug resistance in epilepsy: Clinical impact, potential mechanisms, and new innovative treatment options. *Pharmacological Reviews* **72,** 606–638.

Magloire V, Cornford J, Lieb A, Kullmann DM & Pavlov I (2019). KCC2 overexpression prevents the paradoxical seizure-promoting action of somatic inhibition. *Nature Communications* **10,** 1–13.

Mello LEAM, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL & Finch DM (1993). Circuit Mechanisms of Seizures in the Pilocarpine Model of Chronic Epilepsy: Cell Loss and Mossy Fiber Sprouting. *Epilepsia* **34,** 985–995.

Morris G, Jiruska P, Jefferys JGR & Powell AD (2016). A new approach of modified submerged patch clamp recording reveals interneuronal dynamics during epileptiform oscillations. *Frontiers in Neuroscience*; DOI: 10.3389/fnins.2016.00519.

Morris G, O'Brien D & Henshall DC (2021*a*). Opportunities and challenges for microRNA-targeting therapeutics for epilepsy. *Trends in Pharmacological Sciences* **42,** 605–616.

Morris G, Rowell R & Cunningham MO (2021*b*). Limitations of animal epilepsy research models: Can epileptic human tissue provide translational benefit? *ALTEX*; DOI: 10.14573/altex.2007082.

Morris G & Schorge S (2022). Gene Therapy for Neurological Disease: State of the Art and Opportunities for Next-generation Approaches. *Neuroscience*; DOI: 10.1016/j.neuroscience.2022.03.010.

Mouri G, Jimenez-Mateos E, Engel T, Dunleavy M, Hatazaki S, Paucard A, Matsushima S, Taki W & Henshall DC (2008). Unilateral hippocampal CA3-predominant damage and short latency epileptogenesis after intra-amygdala microinjection of kainic acid in mice. *Brain Research* **1213,** 140–151.

Mula M, Kanner AM, Jetté N & Sander JW (2021). Psychiatric Comorbidities in People With Epilepsy. *Neurol Clin Pract* **11,** e112–e120.

Ngugi AK, Bottomley C, Kleinschmidt I, Sander JW & Newton CR (2010). Estimation of the burden of active and life-time epilepsy: A meta-analytic approach. *Epilepsia* **51,** 883–890.

Norwood BA, Bumanglag A v., Osculati F, Sbarbati A, Marzola P, Nicolato E, Fabene PF & Sloviter RS (2010). Classic hippocampal sclerosis and hippocampal onset epilepsy produced by a single "cryptic" episode of focal hippocampal excitation in awake rats. *Journal of Comparative Neurology* **518,** 3381–3407.

Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, Takeuchi T, Itohara S, Yanagawa Y, Obata K, Furuichi T, Hensch TK & Yamakawa K (2007). Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: A circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. *Journal of Neuroscience* **27,** 5903–5914.

Santana-Gomez CE, Medel-Matus JS & Rundle BK (2021). Animal models of post-traumatic epilepsy and their neurobehavioral comorbidities. *Seizure* **90,** 9–16.

Schwarz N, Hedrich UBS, Schwarz H, Harshad PA, Dammeier N, Auffenberg E, Bedogni F, Honegger JB, Lerche H, Wuttke T v. & Koch H (2017). Human Cerebrospinal fluid promotes long-term neuronal viability and network function in human neocortical organotypic brain slice cultures. *Scientific Reports* **7,** 1–12.

Semple BD, Blomgren K, Gimlin K, Ferriero DM & Noble-Haeusslein LJ (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in Neurobiology* **106–107,** 1–16.

Shi Y, Inoue H, Wu JC & Yamanaka S (2017). Induced pluripotent stem cell technology: A decade of progress. *Nature Reviews Drug Discovery* **16,** 115–130.

van der Staay FJ (2006). Animal models of behavioral dysfunctions: Basic concepts and classifications, and an evaluation strategy. *Brain Research Reviews* **52,** 131–159.

Streng ML & Krook-Magnuson E (2019). Excitation, but not inhibition of the fastigial nucleus provides powerful control over temporal lobe seizures. *The Journal of Physiology*JP278747.

Venø MT et al. (2020). A systems approach delivers a functional microRNA catalog and expanded targets for seizure suppression in temporal lobe epilepsy. *Proc Natl Acad Sci U S A* **117,** 15977–15988.

Vezzani A, French J, Bartfai T & Baram TZ (2011). The role of inflammation in epilepsy. *Nature Reviews Neurology* **7,** 31–40.

Wang Y, Wei P, Yan F, Luo Y & Zhao G (2022). Animal Models of Epilepsy: A Phenotype-oriented Review. *Aging and Disease* **13,** 215–231.
Wenzel M, Hamm JP, Peterka DS & Yuste R (2017). Reliable and Elastic Propagation of Cortical Seizures In Vivo. *Cell Reports* **19,** 2681–2693.

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Data availability statement

N/A

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