Stem Cells and Epilepsy:
Modelling the Brain with Organoids

Daniel Burger

King's College London
public@danielburger.online

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Abstract

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1 Introduction

Epilepsy, a neurological disorder afflicting around 50 million individuals worldwide (World Health Organization, 2019), manifests through recurrent, unprovoked seizures, which can lead to symptoms such as convulsions (i.e. uncontrolled shaking), loss of consciousness, and unusual sensations or behaviours. The development of seizures in epilepsy is mainly due to a complex interaction between the excitatory and inhibitory activities of neurons in the brain. When there is an imbalance that leads to more excitation than inhibition, it can cause sudden and abnormal electrical activity in localised or entire regions of the brain (Robinson et al., 1997).

Temporal lobe epilepsy (TLE) is the most common form (Epilepsy Foundation, 2019)—yet it is the drug-resistant epilepsies, especially those without an identifiable epileptogenic zone, that present the most significant treatment challenges (Iwasaki et al., 2016; Guery & Rheims, 2021). Current interventions, such as neuromodulation (Fisher & Velasco, 2014), offer some hope, but their effectiveness is variable and can be marred by severe side effects, highlighting the urgent need for novel research directions.

The impact of epilepsy extends far beyond its physiological symptoms, affecting every facet of an individual's life. A large number of people who are diagnosed suffer from uncontrollable seizures, rendering them incapable of performing routine tasks, securing employment, or even driving—a prohibition enforced in many countries, such as e.g. Switzerland, due to safety concerns (Schweizerische Epilepsie-Liga, 2021). This pervasive uncertainty cultivates a lifestyle fraught with limitations, emphasising the disease's profound societal and personal toll.

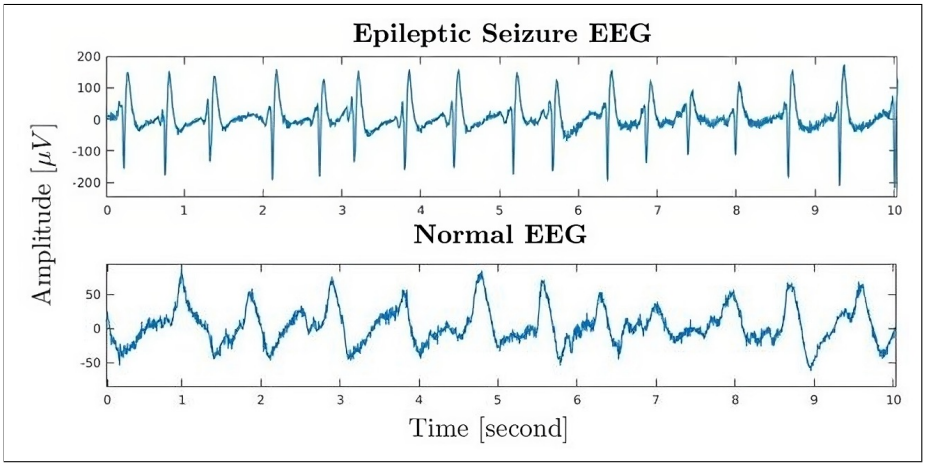

Figure 1.1: EEG Waveforms: Epileptic vs. Normal Brain Activity. Comparative display of EEG waveforms showing brain electrical activity over 10 seconds. The top graph represents EEG data during an epileptic seizure, characterised by high amplitude and frequent spikes, indicative of abnormal neuronal activity. The bottom graph illustrates a normal EEG with regular wave patterns and lower amplitude, reflecting typical brain function. The x-axis measures time in seconds, and the y-axis measures the amplitude of brain waves in microvolts (μV). Image taken from Espinosa et al. (2020).

To fully grasp the essence of epilepsy, one must consider not only its physical manifestations but also the profound psychological toll it exacts on sufferers. The unpredictability of seizures can instil a persistent fear and sense of helplessness, significantly affecting mental health. The origins of epilepsy are diverse, encompassing genetic predispositions (genotype) and observable characteristics (phenotype), including brain injuries and infections, underscoring the complexity of its etiology. The dichotomy between normal and epileptic brain function is starkly illustrated in EEG recordings, as exemplarily shown in Figure 1.1. These visual representations not only highlight the aberrant electrical activity characteristic of seizures but also underscore the critical need for a deeper understanding of the underlying mechanisms of epilepsy. Such insights are essential for paving the way toward more targeted and efficacious treatments, addressing the disease's genotype and phenotype.

1.1 Traditional Models for Epilepsy Research

Traditional epilepsy research models, such as in vivo animal subjects and in silico simulations, have been invaluable yet present notable limitations. Animal models, often e.g. employing rodents (Wang et al., 2022), provide insights but may not fully translate to human epilepsy due to differences in brain structure and function (Kandratavicius et al., 2014). Similarly, in silico models, like those developed by the Blue Brain Project (Markram, 2006), offer detailed simulations of neural networks but are limited by current computational capabilities and understanding of the brain (Mirza et al., 2016).

Stem-cell-derived models, mainly from human induced pluripotent stem cells (hiPSCs), emerge as a promising solution, offering a more accurate and ethical approach to studying epilepsy. Unlike animal models, which face translational hurdles due to species-specific differences in brain architecture and function, hiPSCs can be derived from human cells, ensuring a closer representation of human pathophysiology. Furthermore, the use of hiPSCs sidesteps the ethical quandaries associated with embryonic stem cell research, as they can be obtained from adult cells (e.g. skin cells) without harm to the donor, thereby aligning with ethical standards for human research (Takahashi & Yamanaka, 2006). These models circumvent traditional methods' limitations by providing a renewable source of human neural tissue and enabling the exploration of epilepsy's neurodevelopmental and pathophysiological aspects in a patient-specific context while also allowing the precise analysis and image of the tissues in a highly controlled environment.

1.2 Current State of the Art

The advances in stem cell technology have given rise to another groundbreaking approach in the study of epilepsy: the cultivation of three-dimensional brain organoids derived from hiPSCs. Compared to the first approaches where neuronal cells were cultivated in a two-dimensional manner (sometimes called dish brains), researchers can now generate these cerebral organoids that mimic the brain's complex architectures in terms of tissue structures and the arrangement of cellular types, cell-to-cell interactions, and synaptic connectivity, surpassing two-dimensional dish brains that fall short in replicating the

physiological interactions, regional specificity, and microenvironment gradients observed in vivo (Clevers, 2016; Wang, 2018).

Another benefit of cultivating brain organoids is that it allows researchers to grow or 3D-print neural tissue, e.g. around electrically conductive matrices or electrodes (Yao et al., 2023), engaging with and measuring the organoids in ways reminiscent of living brains. Additionally, combining multiple brain organoids as an assembloid offers a sophisticated method to study the interactions between different brain regions (Sloan et al., 2018). These assembloids recreate the complex neural networks and facilitate investigations into the inter-regional synaptic connections critical for higher-order brain functions, often disrupted in epilepsy.

These state-of-the-art techniques offer a transformative avenue for epilepsy research and beyond. Using hiPSCs to reflect a patient's unique genetic makeup, brain organoids serve as personalised models to decipher the complex interplay of factors driving epileptogenesis. The insights garnered through such increasingly precise 3D models promise to accelerate the discovery of innovative treatments, aiming to improve the quality of life for individuals with neurological diseases.

2 Discussion

2.1 Stem-Cell-Derived Models for Epilepsy

Reprogramming stem cells to model epilepsy in vitro has advanced significantly, allowing researchers to study disease mechanisms and develop new therapies. Although in vitro approaches offer unique opportunities to explore central nervous system (CNS) disorders using patient-derived neural tissue, they still face limitations, such as the difficulty in fully differentiating iPSCs into mature cell types, especially 'aged' cells, which is particularly challenging in age-related brain disorders. However, grafting iPSC-derived neural progenitor cells (NPCs) into embryonic rodent brains allows them to integrate into developing networks and mature in vivo, partially overcoming these limitations (Parent & Anderson, 2015).

Recent findings, such as those from Thodeson et al. (2017), demonstrate the potential of neural stem cells in epilepsy research. These studies have shown that iPSC models can provide novel discoveries in diseases with epileptic phenotypes despite challenges like variable expression profiles and differentiation potential among iPSC lines. Using iPSCs enables the modelling of genetic epilepsies and brain diseases with epileptic symptoms, revealing altered neuronal morphology, spontaneous activity, and ion current density in disease models. For instance, iPSC models of Rett syndrome have shown decreases in neuronal soma size, neurite outgrowth, and synapse formation compared to controls, emphasising the need to consider both neuronal and astrocytic contributions to epileptogenesis.

Evaluating the effectiveness and limitations of stem-cell-derived models, such as those provided by Kandemir et al. (2022), highlights the increased neurogenesis in certain epilepsy models, suggesting the intricate relationship between seizure types and neurogenesis. These models offer profound insights into the pathology of epilepsy and potential therapeutic targets. However, challenges remain, such as ensuring the

specificity of markers for neurogenesis and understanding the role of mature astrocytes expressing markers like DCX, which may not be specific to neurogenesis alone.

2.2 Case Studies and Practical Applications

TODO: - Presenting specific case studies from the references, like the work by Samarasinghe et al. (2021) on identifying neural oscillations in brain organoids and their implications for understanding epilepsy. - KEY PART OF THE ESSAY: Discussing practical applications of these models in understanding and treating epilepsy, referencing Steinberg et al. (2020) and their modelling of genetic epileptic encephalopathies.

Personal note: Main key takeaway from this section is that studying neural tissue in as-close-to-the-real-thing as possible is key. There are more and more methods to really try to get the tissue structure right in order to be able to study the brain in a very controlled environment without ethical issues or lots of efforts compared to finding in vivo tissue. Not only will this research benefit studying it by modelling it, but also by studying it directly via applying medication onto brain organoids and seeing how they react. Other approaches might be creating individualised brain organoids from patients with epilepsy (also called patient-derived induced pluripotent stem cells) and studying their brain tissue in order to find individualised treatments for epilepsy with their specific case. Also, going beyond just studying, one can go one further and then start to treat epilepsy with brain organoids, e.g. by implanting them into the brain of a patient with epilepsy in order to produce GABAergic interneurons in the parts of the brain where they are needed – generally just genetically modifying the implants for the specific patient's disease (referencing upcoming chapter).

2.3 Other Approaches and Advancements

TODO: - Exploring the transplantation of hiPSCs/brain organoids into living beings, referencing Hunt and Baraban (2015) for their work on interneuron transplantation and others. Also mention the work of NRTX-1001 and how they implanted stem cells into the brain of a patient with epilepsy in order to produce GABAergic interneurons in the parts of the brain where they are needed. - Discuss the role and potential of neuroprosthetics in epilepsy treatment and research, drawing on insights from current studies or reviews. - Discuss how multiple brain organoids can be fused into an assembloid to study e.g. neuronal migration from e.g. GABAergic and glutamatergic neurons and how this can be used to study epilepsy and other neurological diseases.

Personal note: Generally the field of neuroprosthetics with synthetic biological neural tissue is quite interesting and also a personal interest of the author. Not only does it go into the field of personalised treatment (also sometimes called precision medicine) and medicine but also into the field of synthetic biology and bioengineering. Cite text from "Augmenting Cognition" book from Markram, where Mijail Demian Serruya writes: "3.9 Expanding the neural substrate Instead of using pairs of recording and stimulating arrays to reconnect one cortical area to another within a patient's brain, one could consider routing the signals through an artificial model of the cortex. If this were possible, one could provide patients with additional neural substrate.Just as regions of the brain may be become unusable due to stroke, injury or degenerative

conditions, so too there might be the possibility to add new virtual or ectopic cortex to compensate for lost tissue. Recordings of units throughout the brain could be fed into a software model, or into actual ectopic neural tissue, and then activity from this model or neural tissue could be used to trigger stimulation back into the patient's brain (Fig. 3.3c). Initially, such additional cortex could exist as computational models in software programs bidirectionally linked to a patient through wireless connections. Eventually, software models could be rendered in hardware as an encapsulated silicon chip, e.g., neuromorphic very-large-scale-integrated (VLSI) microchips, that in turn could be implanted in the body."

2.4 Ethical Considerations

TODO: - Discussing ethical issues surrounding stem cell research and brain organoid models, citing Farahany et al. (2018) for a comprehensive view of the ethics of experimenting with human brain tissue. - Address specific ethical questions, such as the consciousness of brain organoids and the moral implications of in vitro experimentation.

Personal note: Generally focus on the aspect of scaling brain organoids. Currently limited by the blood supply (still, ongoing research and most likely being solved in the coming years) and the issues that we don't know how to accurately model the tissue structure of an actual human brain, which is most likely necessary to create a consciousness human brain. However, as mentioned before, both of these issues are being worked on as their promises are huge. Therefore the issue of creating sentient/conscious brain organoids in the labs is a topic of ongoing debate and research. Also mention the company FinalSpark in Switzerland, that e.g. uses brain organoids for running AI algorithms and how this is a very promising field of research and how it is important to keep an eye on the ethical implications of this research, as we don't want to create sentient beings in the lab without knowing it and as slaves for AI algorithms (e.g. creating a sentient being that is constantly in pain and suffering, as it is used for running a specific AI algorithm).

3 Conclusion

TODO: - Summarising the key points, emphasising the impact of stem-cell-derived models compared to other models, especially e.g. in silico or animal testing. - Discussing future prospects of stem-cell-derived models in neuroscience, considering technological advancements and potential breakthroughs. - Addressing remaining challenges, including technical, ethical, and funding-related issues, to give a balanced view of the field's future.

Personal note: Basically conclude and have a key focus on that this field needs to get more funding, as brain organoids coupled with personalised medicine (also sometimes called precision medicine) and gene-editing is a very promising field of research and has the potential to cure epilepsy and other neurological diseases. Also, mention somehow neuroprosthetics again the the whole point from Mijail Demian Serruya's part of the "Augmenting Cognition" book from Markram.

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