Stem Cells and Epilepsy:
Modelling the Brain with Organoids

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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.

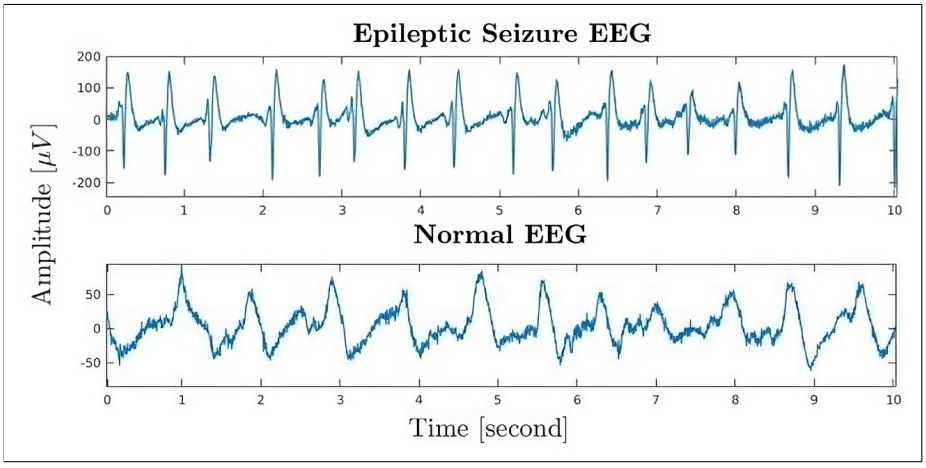

Figure 1.1: EEG Waveforms: Epileptic vs. Normal Brain Activity. Comparative display of EEG waveforms showing brain electrical activity over 10 seconds. The top graph represents EEG data during an epileptic seizure, characterised by high amplitude and frequent spikes, indicative of abnormal neuronal activity. 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

One of the most compelling aspects of hiPSC technology is its ability to model genetic forms of epilepsy. By deriving iPSCs from patients with known genetic mutations, researchers can observe the direct effects of these mutations on neuronal development, function, and network formation. This approach has been instrumental in elucidating the pathophysiological mechanisms underlying syndromes such as Dravet Syndrome and Tuberous Sclerosis Complex, where specific gene mutations lead to distinct epileptic phenotypes (Jiao et al., 2013; Nadadhur et al., 2019).

Despite these advances, stem-cell-derived models of epilepsy face several challenges. The differentiation of iPSCs into fully mature, functional neurons and glial cells that accurately represent the diversity and complexity of the human brain remains a daunting task. This is particularly pertinent in modelling age-related epilepsies, where the disease phenotype may only manifest or worsen over time. As mentioned earlier, techniques such as 3D brain organoids have emerged as a promising solution to mimic the brain's architecture and cellular heterogeneity more closely, though they are not without their own set of limitations, including the lack of vascularization and an immune system (Lancaster et al., 2013; Di Lullo & Kriegstein, 2017).

Recent studies, such as those by Thodeson et al. (2017), underscore the potential of neural stem cells in epilepsy research. These investigations reveal that iPSC models can uncover novel insights into diseases with epileptic phenotypes, despite challenges such as variable expression profiles and differentiation potential among iPSC lines. For example, iPSC models of Rett syndrome have illuminated critical aspects of epileptogenesis by demonstrating decreases in neuronal soma size, neurite outgrowth, and synapse formation compared to controls, thereby highlighting the intricate interplay between neuronal and astrocytic contributions to the disease (Marchetto et al., 2010).

The evaluation of stem-cell-derived models' effectiveness and limitations, as discussed by Kandemir et al. (2022), points to the nuanced relationship between neurogenesis and epilepsy. These models not only provide deep insights into the pathological underpinnings of epilepsy but also illuminate potential therapeutic avenues. However, the specificity of neurogenesis markers and the role of mature astrocytes in epilepsy remain areas of ongoing research, emphasizing the need for continued innovation and refinement in stem-cell-derived epilepsy models (Jessberger & Parent, 2015).

2.2 Case Studies and Practical Applications

Building on the foundational concepts discussed earlier, we now shift our focus to two pivotal case studies and practical examples. These instances illuminate the real-world application of stem-cell-derived models in epilepsy research, showcasing the depth of insights gained and the potential pathways to therapeutic advancements.

- **Samarasinghe et al. (2021)** delved into the complex neural dynamics of brain organoids, uncovering significant epileptiform activities within organoids modeling Rett syndrome, which is a genetic disorder that typically affects females and is characterised by impairments in language and coordination, repetitive movements, slower growth, difficulty walking and so on. Complications can include seizures, scoliosis, and sleeping problems. However, Samarasinghe et al. groundbreaking work demonstrated not only the presence of sophisticated physiological activities within these organoids but also the potential for therapeutic intervention. Remarkably, they observed a substantial reduction in epileptiform activities upon administration of pifithrin-α, a TP53 inhibitor, which points towards new directions in epilepsy treatment, especially for conditions exhibiting resistance to conventional therapies.
- **Steinberg et al. (2020)** embarked on an ambitious project to model epileptic encephalopathies using a combination of CRISPR-engineered human ES cells and patient-derived iPSCs, with a focus on the devastating WOREE syndrome. Their meticulous approach unveiled significant cellular and molecular CNS abnormalities, including alterations in GABAergic markers which suggest a disruption in the development of normal and balanced neuronal networks. This work not only underscores the intricate pathophysiology of epileptic encephalopathies but also provides a proof-of-concept for potential therapeutic interventions, such as the modulation of GABAergic responses, which are crucial in the dynamics of developmental epilepsies.

These case studies emphasize the critical importance of accurately mimicking human brain tissue in research to provide a platform for studying epilepsy in a controlled, ethical manner. The advancements in stem-cell-derived models, particularly organoids, open up new avenues for direct therapeutic research and personalized treatment strategies. By enabling the study of individualized brain organoids from patients with epilepsy, researchers can explore tailored treatments for specific cases, thereby enhancing the efficacy and precision of epilepsy management. Furthermore, these models hold promise for innovative treatments, such as the potential use of organoids for cell-based therapies in epilepsy, by genetically modifying implants to address the unique needs of each

patient's condition.

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. 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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