THESIS SYNOPSIS

The human brain is a highly evolved structure responsible for many aspects of cognitive function and is affected in numerous neurological disorders. Human neurogenesis is a particularly sensitive period, where defects in ion channel expression, synaptic formation, and neural stem cell proliferation are linked with neurological disorders. Researchers have for long studied neurological disease pathogenesis in nonhuman species to improve treatments and cure disease, however the aetiology is still unknown in 80% of cases. Given the disparity between human and nonhuman species across neurogenesis mechanisms, differences are also found in the functionality, morphology and biophysiological properties of cortical neurons. This suggests a need for *in-vitro* models which recapitulate human neurogenesis and human-specific electrophysiology to subsequently improve bench-to-clinic translation.

Advances in cellular reprogramming have made it possible to differentiate human induced pluripotent stem-cells (hiPSC) into monolayer or self-organizing three-dimensional (3D) cellular ensembles which recapitulate key features of neurogenesis and a patient's genetic landscape. Remarkably, studies have demonstrated their ability to generate functionally mature excitatory and inhibitory neurons, along with nested oscillatory dynamics. Researchers have therefore utilized hiPSC-derived neuronal models to link electrophysiological properties with underlying biological mechanisms in a healthy and disease context. However, the functional relevance of hiPSC-derived neurons is largely unknown given the lack of comparison with *ex-vivo* human brain tissue at the cellular-level. Moreover, tissue-culture conditions pertain an unphysiological environment which hinders neural activity and introduces photo-toxic affects following functional imaging and optogenetic applications. This thesis comprises five main chapters, covering five main topics: 1) the evolution of cortical neural circuits and neuronal subtypes, 2) methodologies for patch-clamp recordings in hiPSC-derived neurons. 3) optimizing physiological tissue culture media *in-vitro* for optogenetic and functional imaging applications, 4) comparing intrinsic firing properties between hPSC-derived neurons and *ex-vivo* human biopsy cortical tissue, and 5) recapitulating human paediatric epileptic signals using brain organoids.

Chapter 1: The brain of modern humans is disproportionally expanded compared with mouse. Such evolutionary conserved mechanisms have rendered the human brain unique in its total volume, neuron numbers, and proportions occupied by supragranular layers. Subsequently, recent studies have emerged evaluating the morpho-electric and transcriptomic profiles of human cortical neurons, revealing human-specific neural subtypes. In chapter 1, I review recent findings on the distinct structural, functional and trancriptomic features of human cortical neurons. In addition, I outline the mechanisms of neurogenesis and epileptogenic activity, and summarise current human iPSC-derived neuronal models. Together, chapter 1 outlines key-concepts and background information for the remaining thesis chapters.

Chapter 2: Patch-clamp recordings from hiPSC-derived neurons are the gold-standard for functional intrinsic cell-feature evaluations. However, methodologies specific to *in-vitro* neuronal models are largely under-represented in the literature. Moreover, current methods fail to address unphysiological tissue culture conditions, phototoxicity, and heterogenous *in-vitro* electrophysiology. Therefore, we review available biotechnologies and provide practical tips, data acquisition techniques and analysis pipelines to address these common issues. Together, these recommendations serve as a guide for electrophysiologists acquiring and analysing patch-clamp recordings from hiPSC-derived neuronal cultures models. ***This work was accepted in 2023 (March) as a contributing chapter titled 'Patch-clamp recordings from human induced pluripotent stem cell-derived neurons' to the textbook 'Practical guide to electrophysiology for neurophysiologists' by World Scientific Publishing.***

Chapter 3: The capabilities of functional live imaging technologies, fluorescent sensors, and optogenetics tools in neuroscience are advancing. In parallel, cellular reprogramming are expanding the use of human neuronal models *in vitro*. However, high intensity fluorescence excitation can damage living organisms via complex wavelength-dependent photophysical mechanisms in a term referred to as 'phototoxicity'. Current tissue culture media (e.g. NUEMO, FluoroBrite) introduce phototoxic compounds, suboptimal fluorescence signals, and confer unphysiological electrical activity. Therefore, this highlights a need for tissue culture media better adapted to live-cell imaging with the added utility of supporting physiological neuronal electrical and synaptic activity *in-vitro*. To overcome these issues, we designed a neuromedium called BrainPhys™ Imaging (BPI) to improve the quality of a wide range of fluorescence imaging applications with live neurons *in vitro* while supporting optimal neuronal viability and function. ***This work was published in Nature Communications (IF = 18) in 2020*** (https://doi.org/10.1038/s41467-020-19275-x). *Moreover, it was featured as an editor highlight in March 2021 and received a 'best publication by a HDR student' award in 2021 by Flinders University.*

Chapter 4: hiPSC cell-derived models represent a powerful tool for studying human neurological disorders, especially those with physiological and network abnormalities. However, whether the functionality of mature hPSC-derived neurons is comparable to the human brain itself is unknown at the single-cell level. To address this, I developed a computational toolbox (BrainSpike) to extract intrinsic cell features and classify functional states of neuronal cell types using machine-learning driven approaches. Using BrainSpike, patch-clamp recordings from both 331 cortical hiPSC-derived neurons and 685 surgically resected human cortical neurons (across 6 laminar layers and 13 anatomical regions) were systematically compared. To probe functional and transcriptomic correlates, patch-sequence ('Patch-seq') datasets were integrated to map human-specific molecular subtypes. Greater than 120 days *in-vitro*, subpopulations of hPS cell-derived cortical neurons emerged to share similar functional properties with deep layers of the human cortex enriched in human-specific markers (*COL22A1*). *This work is being prepared for submission.*

Chapter 5: Childhood epilepsy syndromes begin with a phase of progressive network dysfunction in the developing brain culminating in epileptic seizures. Despite high frequency oscillations (HFO) serving as biomarkers for identifying epileptogenic tissue, understanding epileptogenesis has been challenging due to the lack of accurate models for prenatal human brain network development and dysfunction. In this chapter, we use human iPSC-derived cerebral organoids to uncover the emergence of epileptogenic networks in tuberous sclerosis complex (TSC). Extracellular recordings from TSC and isogenic control cerebral brain organoids were provided by the Knoblich Lab (IMBA; Vienna, Austria). Subsequently, I developed a computational toolbox for HFO signal feature extractions to robustly quantify pathological HFOs. Results show that features of epileptogenic regions like frequent population network discharges and pathological HFOs, along with pathological interactions between HFO and low frequency oscillations (delta and theta), were recapitulated by *in-vitro* TSC networks. Comparisons with intraoperative electrocorticography recordings from TSC patients (provided by the Ziljman Lab; Utrecht University; Utrecht, Netherlands) showed that resecting tissue generating epileptogenic features recapitulated in organoids lead to seizure freedom. Pathological network development *in-vitro* was caused by a specific type of caudal interneurons and caused hyperexcitability-related morphological changes. *This work is being prepared for submission.*

Overall, this PhD project aims to expand our understanding of the intrinsic functional features of human PS cell-derived neuronal models, and their ability to recapitulate key human functional properties. Furthermore, the electrophysiology software and analysis pipelines generated in this project serve as a platform for widespread use and can be applied to other disease and health contexts. The work undertaken in this PhD benefits all laboratories relying on hPSC-derived neuronal models to study human neurological disorders, especially with network abnormalities.