IST Austria Postdoctoral Fellowship

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Research statement

Epilepsy is a devastating disease that affects millions of people worldwide. Modern gene typing allows us to identify the genetic background of this burdensome disease but understanding the influence of genetic differences on seizure dynamics in human cortical networks remains difficult, not least because of the ethical complexities of clinical neuroscience.

Recently, it has become possible to grow cultures of human cortical networks *ex vivo* and obtain the activity at single-cell resolution over extended periods of time. Here, I propose to develop the computational and theoretical tools to analyse the emerging dynamics in human cell cultures and compare them, for the first time, to the long-standing theoretical models of excitatory and inhibitory neural networks.

This collaborative work will help to reveal the differences between natural and disease dynamics and contribute to effective life-long clinical treatments.

In detail, I seek to address the following aims:

- Aim 1: Establish a pipeline for spike sorting, analysis, and identification of neuronal phenotypes in human induced pluripotent stem cell (iPSC)-derived cultures.
- Aim 2: Compare neuronal iPSC-derived culture dynamics with theoretical models and, importantly, adjust our current models to reflect more accurately the observed data.
- Aim 3: Investigate, by way of perturbation experiments and large scale network modelling, how activity-dependent plasticity mechanisms in developing cultures contribute to matured networks.

1. Establish a pipeline for spike sorting, analysis, and identification of neuronal phenotypes in human induced pluripotent stem cell (iPSC)-derived cultures.

The ability to record from multiple neurons simultaneously has grown exponentially with the development of high-density multi-electrode arrays (HD-MEA). Today, HD-MEAs can have tens of thousands of recording sites able to capture the simultaneous activity of thousands of neurons, often capturing the detail at single-neuron resolution (1). To analyse the activity of these cultures, the raw data from the HD-MEA must be processed through a technique known as "spike sorting". Spike sorting is a broad spectrum of methods that may include custom-developed pattern-matching of an electrophysiological profile, the use of convolutional neural networks to construct these templates automatically, and the extraction of the spike times of each event in the culture (2; 3). Although computationally expensive, the development of fast and robust spike sorting methods like KiloSort2 has promoted the analysis of dense MEA data.

By accessing single-unit activity profiles of thousands of neurons simultaneously, the contribution of individual neurons to the network activity "whole" can be analysed. Previously, multiple experimentally complex techniques were used to connect the scales of neurons and networks via patch-clamping of a few neurons and recording the local electric field potential of an area of neurons, often at separate time points. Now, the coupling of advanced hardware with computational techniques promises a clearer picture of multiple neuronal scales at once, but existing solutions are not easily portable to answer new questions.

Different neuron types tend to exhibit distinct electrophysiological characteristics that can be identified by their voltage activity profile (4). The advancement of human induced pluripotent stem cell (iPSC)-derived cultures has allowed the controlled development of biological neural networks for close inspection (5). Indeed, the technique is poised to become the state-of-the-art method for non-invasive exploration of human neuronal activity for clinical guidance of neuropathologies such as epilepsy and autism spectrum disorder. Fortunately, the recording of neuronal human iPSC-derived cultures using HD-MEAs is established at the Institute of Science and Technology (IST) Austria, one of the only places in the world to set this up due to the technical challenges involved. A limiting factor in realising its potential, however, is the scalability of the current technology. Currently, while the experimental methods are being improved and refined, the supporting software infrastructure remains a piecemeal of disparate solutions. To support the scaling of neuronal human iPSC-derived cultures for diagnostic and therapeutic use, I propose the development of an efficient easy-to-use software pipeline. Along with clinical relevance, this project will be scientifically valuable to all parties.

The pipeline will connect the raw HD-MEA data with algorithms such as spike sorting and network analysis tools. Notably, the database of recordings can be linked with anonymised patient records that can include molecular genetic markers. While the research will focus on epilepsy and autism spectrum disorder patients, the solution will have the potential to branch to other pathologies. Furthermore, I will be able to easily extend the database to include further details such as pharmacological interventions. By connecting these electrophysiological, genetic, and pharmacological data through an integrated software system, I can explore vital links between the data for diagnosis and potential therapeutics. Building an integrated solution allows the smooth scaling of the data to more patients as the experimental process is also scaled.

I am an experienced scientist with industrial knowledge of building software systems for large companies (Comair). I also have experience with genetics (from my BSc Hons in Physiology), neuroelectrophysiology (from my MSc in Neuroscience), and data analysis (from my PhD in Computational Neuroscience). Prof Tim Vogels, the host supervisor, has overseen multiple development pipeline projects including ICGnealogy and WorldWideNeuro. This project requires close collaboration with Prof Gaia Novarino from IST Austria and Dr Carsten Pfeffer from the Technology Transfer @ IST Austria (TWIST). With their extensive expertise in developing and recording human iPSC-derived cultures, along with their advanced analysis of genetic markers, I can build a novel solution to advance the cutting-edge of neuronal human stem cell science.

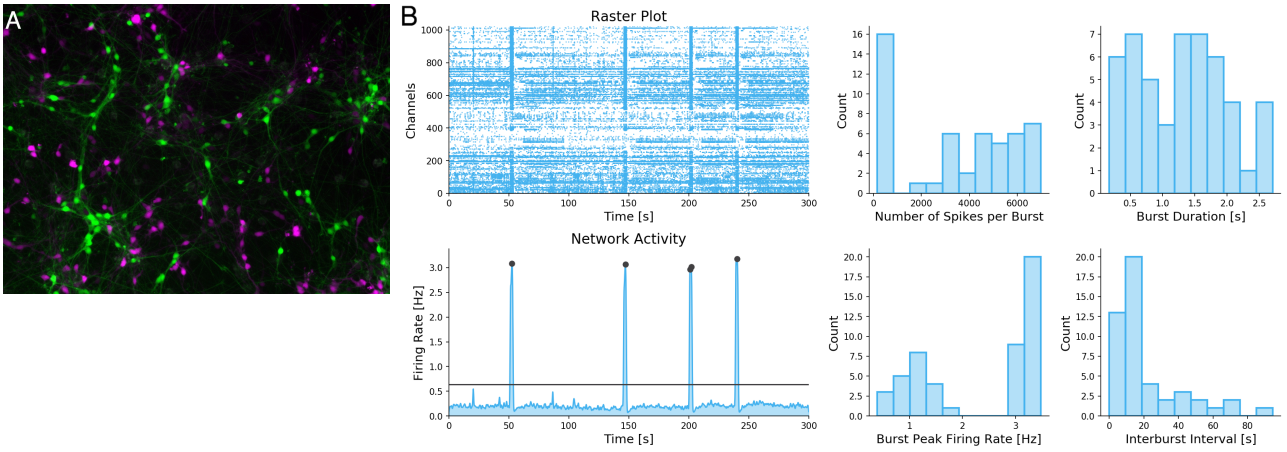

Figure 1: Growth and recording of neurons derived from human iPSCs using methods established at IST Austria.
A, coculture of human iPSC-derived excitatory (green fluorescence) and inhibitory (magenta fluorescence) neurons cultured for 2 weeks.
B, HD-MEA recording of neuronal activity from human iPSC-derived coculture of excitatory and inhibitory neurons together with rat astrocytes after 5 weeks.
Images courtesy of Dr Carsten Pfeffer.

2. Compare neuronal iPSC-derived culture dynamics with theoretical models and adjust the models to reflect more accurately the observed data.

Theoretical neuroscientists have studied neural network activity for decades by assimilating multiple pieces of experimental data and incorporating the results into computational models. By virtue of the difficult experimental setups, the models have often relied on separate pieces of evidence to support the model's structure. Furthermore, some heuristics have become prevalent throughout the field, such as random connectivity, an excitation-inhibition ratio of 80-20 and homogenous populations of neurons, and asynchronous dynamics. Although computationally tractable, the abstraction of biological complexity increases the distance to experimental results and thus biological relevance. With the advent of large-scale neural recordings, I can compare the common assumptions in computational models against the rich multi-faceted data from HD-MEAs for the first time without ignoring important details.

By accessing the activity of thousands of neurons simultaneously, I can examine in detail both individual profiles of single units as well as population dynamics (see aim 1). Modern computational neuroscience frameworks enable the modelling of thousands of neurons at an unprecedented level of detail. These frameworks also commonly support graphical processing units (GPUs) or high-performance computing (HPC) clusters for the scaling of network dynamics to many thousands of neurons. From these frameworks, large biological neural networks have been built to mimic and extend experimental results, generating hypotheses and providing further insight into processes such as learning, memory, and disease. Through leveraging large-scale datasets such as those from HD-MEA recordings, common model architectures like the balanced excitatory-inhibitory network can have its biological realism explored and updated. For example, recording the number of each type of neuron in iPSC-derived cultures can inform the network definition of an *in silico* model that recapitulates the functional behaviour. Fundamentally, many biological models have homogenous populations of neurons. With large-scale access to the activity of single neurons embedded within an active network, I can build a network model composed of individually unique neurons. Previous work was limited by the number of simultaneous neurons that could be precisely recorded. Now, I can build computational models that are informed by holistic biological data at an unprecedented scale. As an extra step, my expertise in ion-based changes to network dynamics enables the unique activity profiles of neurons to be linked to underlying ion changes that are experimentally complex to record.

Recent advances in machine learning have enabled the "hands-free" exploration of parameters that is less computationally intensive than the intractable exploration of the full parameter space. Furthermore, techniques are continually being developed to fit more accurately computational models to experimental data (6; 7). However, the scale of comparison between biological and computational neural network is often limited to individual neurons or aggregated population behaviour. I propose the novel development of techniques to build, compare, and fit computational models that are informed by HD-MEA recordings of thousands of neurons. By leveraging the physiological detail enabled by aim 1, the computational model can have individual neurons as well as the overarching network dynamics fitted against data. The multi-scale fitting will be a novel development that builds upon existing fitting methods. Crucially, I will be the first to leverage machine learning to understand neurons in human iPSC-derived cultures.

Importantly, the models will be informed by human neurons that can be compared to mouse neurons to identify significant differences between species as well as update computational models to reflect human network dynamics. With the goal of most animal research in neuroscience to understand the *human* brain, this work can yield critical differences between human and animal brains. Human tissue samples have been historically challenging to analyse due to the invasiveness of harvesting tissue usually through elective surgeries for severe neurological conditions. Although neurosurgeons resect some healthier tissue along the path to a pathogenic focal area, the tissue is ultimately from a person with a neurological condition who needed surgery. By utilising iPSC-derived cultures, I can non-invasively access tissue from both patients with disease and controls such as patients' siblings. With access to both healthy and diseased tissue, I will develop computational models that reflect both dynamics depending on measured genetic differences. The computational models could inform future pharmacological interventions within tissue cultures and potentially, eventually, in the clinical setting.

The collaborators for this project, including Prof Gaia Novarino and Dr Carsten Pfeffer at IST Austria, are specifically focused on patients with epilepsy and autism spectrum disorder. To complement this focus, I have broad experience modelling thousands of neurons in a model of status epilepticus, a severe type of epileptic seizure, during my PhD. Furthermore, I gained valuable expertise in the modelling of balanced neural networks during my research visit to Prof Henning Sprekeler's lab at the Technische Universität Berlin and ground my models with experimental data from Dr Joseph Raimondo's lab at the University of Cape Town. Finally, Prof Tim Vogels is an expert in balanced network models of excitation and inhibition, who will lend valuable advice and guidance.

This aim seeks to build data-driven biological neural network models from human iPSC-derived cultures, which will fundamentally guide future model development. This work is enabled by the expertise accessible at IST Austria, coupled with my unique background in experimental neuroscience, computational modelling of biological networks, machine learning, and industrial software engineering.

3. Investigate how structural plasticity mechanisms in developing cultures contribute to functional networks.

The brain has developed multiple, dynamic, interacting, mechanisms to change the strength of connections between neurons (synapses) in order to be able to process, learn, and memorise our environment. A large variety of these mechanisms, "synaptic plasticity rules", have been observed experimentally, yet require substantial and prolonged experimental control. Due to their experimental difficulty, computational neuroscience has played an essential role in providing insight into how certain rules can lead to specific network architectures and elaborate functional dynamics. Plasticity is required for normal brain function and is particularly relevant during neuronal development for the initial formation and stabilisation of connections. Its disruption has important clinical implications, having been linked to pathologies such as Parkinson's disease, epilepsy, and autism spectrum disorder (8). Understanding in detail the multiple synaptic rules that link the architecture and function of a neural network can therefore provide valuable insight into how we think and how "plasticity pathologies" manifest. I propose the use of HD-MEAs for the recording of developing neuronal networks in human iPSC-derived cultures, along with genetic manipulation tools, to provide unprecedented access to understanding synaptic plasticity rules.

Change in synaptic strength has been investigated for decades in both experimental and theoretical setups. Experiments have traditionally focused on single neuron synaptic changes due to technical limitations. Theoreticians have been able to explore larger networks but with a restricted subset of plasticity mechanisms at play. However, little is known about how multiple neurons and synapses may coordinate their changes or react to concurrent activity. Through the recording of 1000s of neurons simultaneously using HD-MEAs, I can develop a more accurate picture of plasticity in the working brain. Although using iPSC-derived cultures is not the same as an awake, behaving animal, it does provide a broad range of possible manipulations to investigate this important phenomenon. Crucially, the maturation of neurons and synapses can be recorded over hours, days, and weeks to understand the emerging plasticity dynamics. Furthermore, this will be done using human iPSC-derived cultures. There is already some evidence that classical plasticity rules established from rodent models have different human analogues (9). By using human iPSC-derived cultures, I can explore these plasticity rules at a hitherto unexplored level of detail.

Pathologies arising from disrupted plasticity are also prime targets to be explored using HD-MEAs recordings of neuronal human iPSC-derived cultures. With access to pathological human samples as well as healthy controls, I can explore the differences in plasticity dynamics to provide insight and potential therapeutic targets. By interfacing between experimental data and computational models, I can further elucidate the functions of developing networks.

The genetic manipulation of animal models has provided valuable insight into plasticity mechanisms (10). For example, genetically modifying certain neurons to respond to specific wavelengths of light – "optogenetics" – allows the selective manipulation of neural activity to identify structural connections and functional circuits. Other genetic manipulations include knocking out or inserting a specific gene (e.g. using CRISPR (11; 12)) and selectively activating receptors using designers drugs (DREADDs (13)). Many of these genetic tools can be exported from animal models to human iPSCs

for investigating human plasticity mechanisms in an active, directed approach.

By collaborating with researchers at IST Austria, we together have the unique ability to investigate plasticity rules in human neural networks over extended periods with the possibility of direct intervention. This work is complemented by Prof Tim Vogels' current project that involves using machine learning to determine functional plasticity rules. Here, I can combine for the first time machine learning and stem cell research for understanding plasticity in human brains. Using advanced genetic and computational techniques at our disposal, I aim to determine how neurons connect to form functional networks. With this knowledge, I can build more accurate models of the brain that can process, learn, and remember. Crucially, insight into plasticity within diseased tissue – e.g. from epilepsy and autism spectrum disorder patients – can further our collective understanding of complex neurological disorders that may inform future treatments.

This interdisciplinary project is possible because of the physiological and genetic expertise from collaborators at IST Austria - Prof Gaia Novarino's lab and Dr Carsten Pfeffer at TWIST. Combining their experimental knowledge with Prof Tim Vogels' global leadership in computational plasticity rules creates a distinctively suitable environment for this project. In addition, my work with plasticity mechanisms in status epilepticus (during my PhD Neuroscience) lends itself well to a project that requires advanced knowledge of plasticity in normal and diseased networks. My complementary machine learning skillset and Prof Vogels' concurrent "machine learning and plasticity" project further motivates for me to pursue this work.

Summary

This project will use software engineering, machine learning, and computational models to build upon the experimental advances in HD-MEAs and human iPSC-derived cultures to understand both normal and diseased brains further. The impact of this work will extend from neuro-electrophysiology to computational models of the mind. Crucially, results will inform clinical neuroscience for plasticity pathologies such as epilepsy and autism spectrum disorder. The project is carefully structured to be both foundational and scalable. The final aim of determining plasticity rules in human networks, at an unprecedently large scale, uses the foundational data analysis pipeline in aim 1 and the experimental-theoretical model comparisons established in aim 2. Leveraging my scientific and software engineering expertise, I constructed each aim to be robust, repeatable, and scalable to further use-cases. This work is only possible in an environment like IST Austria that encourages cross-collaboration.

Along with the personal motivation separately attached, my expertise uniquely suits this project that extends the cutting-edge of neuronal stem cell research by using computational methods.

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