CATAPULT Medicines Discovery

Methods to Examine Neuronal Morphology and Function in Human iPSC-derived Disease Models

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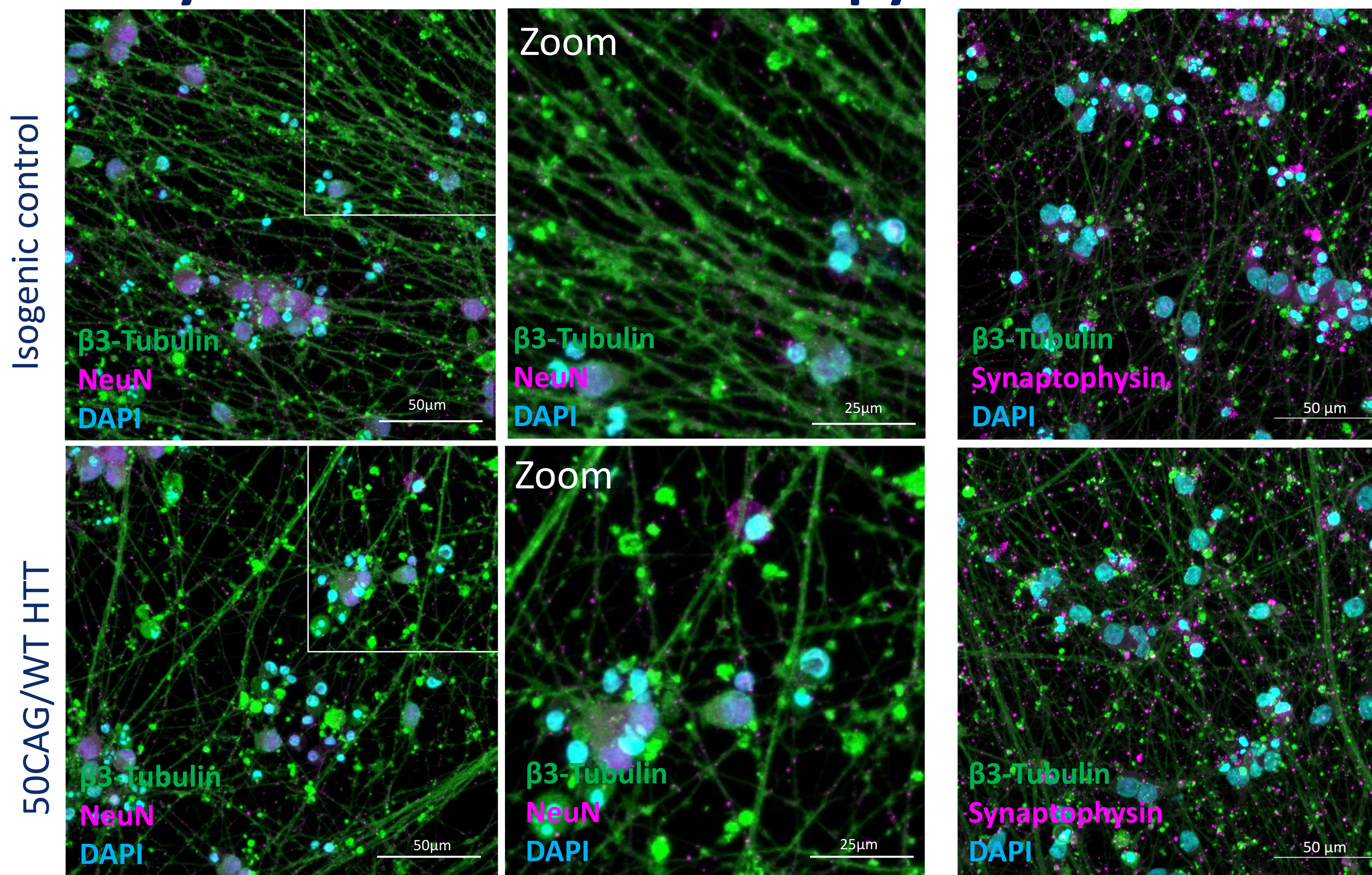
Introduction

- The ability to reprogram somatic cells to stem cells has revolutionised the modelling of human diseases *in vitro*
- **Induced pluripotent stem cells** (iPSCs) can be used to generate multiple cell types found in the central nervous system (CNS), including various types of neurons, astrocytes, and microglia
- Mutations can also be introduced to model monogenic diseases, for example using CRISPR-Cas9. This is particularly advantageous as it allows the comparison of molecular and cellular disease phenotypes against the same isogenic control background
- At MDC, we use the techniques outlined in this poster to examine **morphological and functional changes** that occur due to disease mutations or drug treatments
- Here we present these techniques using an iPSC-derived neuron model from bit.bio that has been engineered to have the mutation associated with **Huntington's disease** (HD), as an example of a specific disease model
- Huntington's disease is a monogenic disease caused by the inheritance of a poly-glutamine repeat expansion in the huntingtin (HTT) gene, encoded by a repeated cytosine-adenine-guanine (CAG) sequence
- Between 36 and 40 CAG repeats causes incomplete penetrance of the disease, whereas patients with **more than 40 CAG** repeats will go on to develop the disorder during a normal lifespan
- The bit.bio HD model used at MDC has a **50CAG repeat expansion** inserted using CRISPR (50CAG/WT HTT)

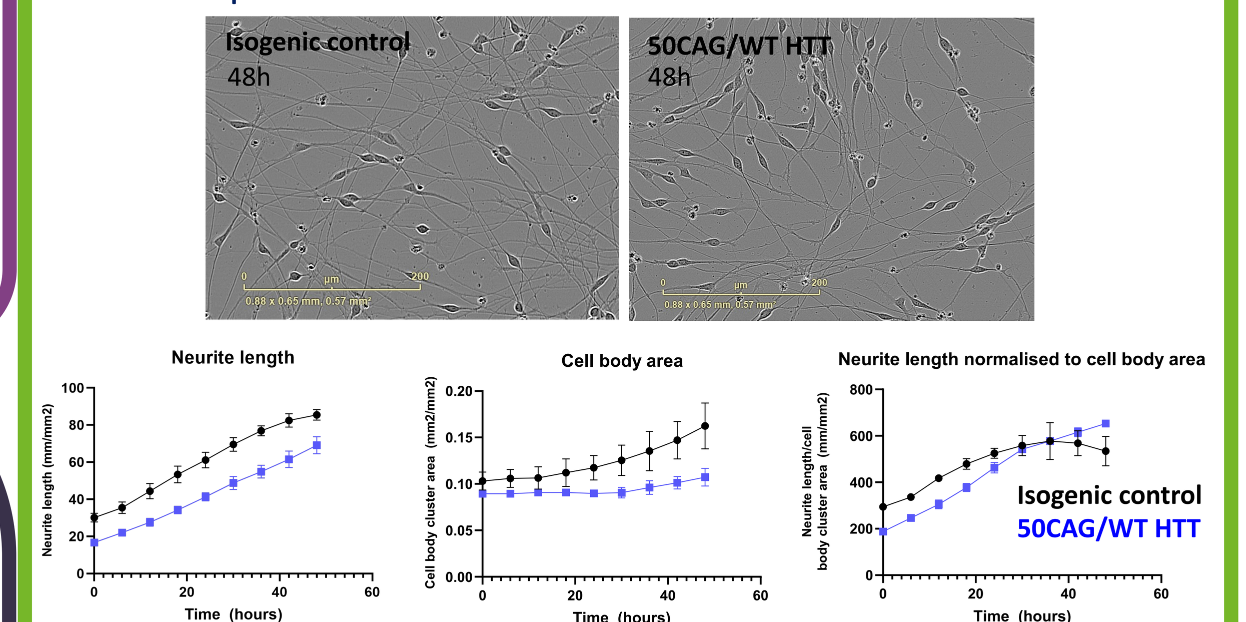
Immunocytochemistry and Confocal Microscopy

- Morphological changes associated with a disease phenotype such as HD can be probed using structural markers such as β3-Tubulin or NeuN, or synaptic markers such as synaptophysin
- Nuclei morphology and count can be used as a read-out of cell viability
- The ability of compounds to rescue this phenotype can then be examined
- Images were taken with a Zeiss LSM880 confocal

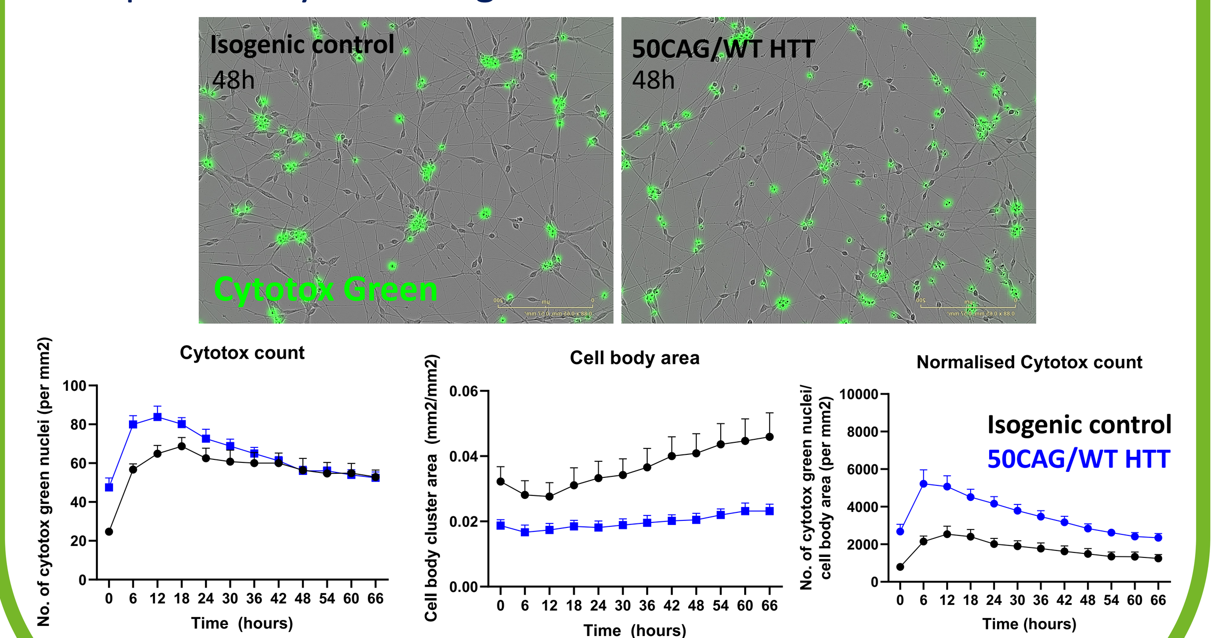


Neurite Outgrowth and Viability

- Live-cell Incucyte imaging can give morphological and neurotoxicity information over time, which can be particularly informative in neurodegenerative disease modelling and drug discovery
- Reduced neurite outgrowth following plating can be a phenotype of neurodegeneration – preliminary results suggest slower neurite outgrowth in HD neurons compared to control

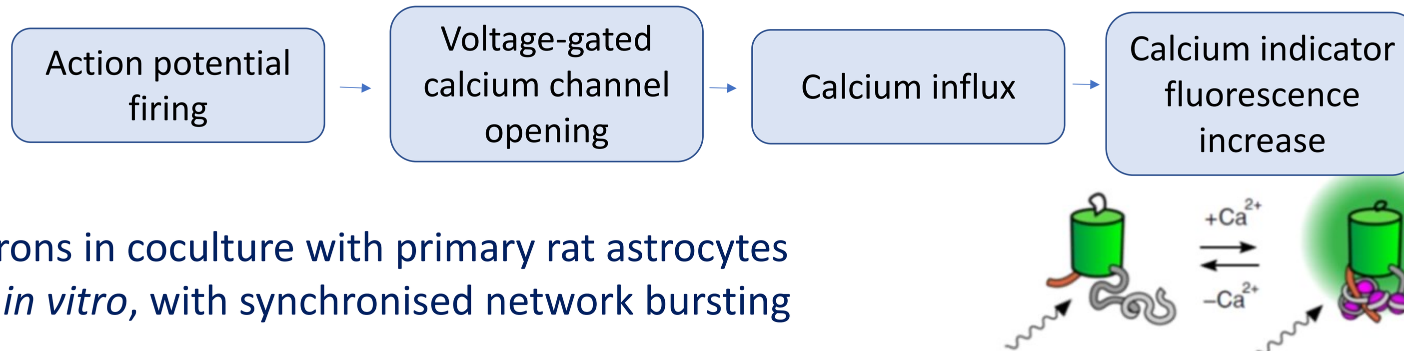


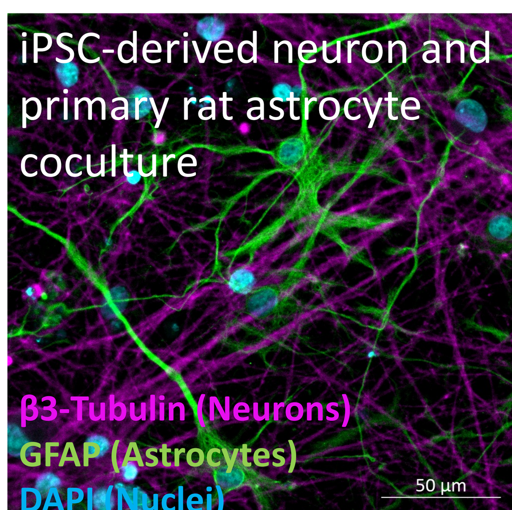
- Use of dyes like Cytotox green that detect cells with disrupted, permeable membranes can be used to determine cell survival and toxicity in disease models
- Preliminary results suggest that HD neurons have decreased viability compared to isogenic controls, potentially reflecting a toxic effect of mutated HTT



Neuronal Activity Assays

- Intracellular calcium concentration fluctuations can be quantified using calcium sensitive indicators as an indirect measure of action potential firing in neurons
- Isogenic control and 50CAG/WT neurons in coculture with primary rat astrocytes begin to fire action potentials at 20 days *in vitro*, with synchronised network bursting activity detected at 25 days

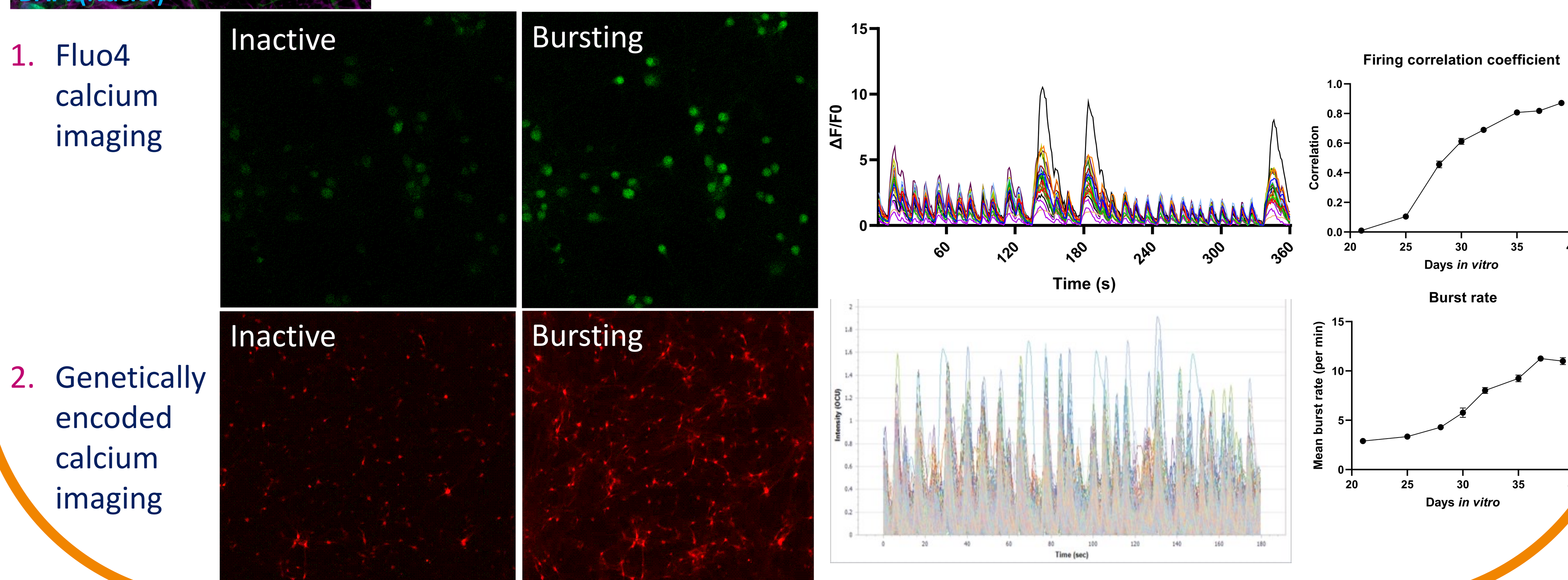




- Calcium indicators can be introduced either by loading neurons with cell-permeant dyes, or by lentiviral transduction of genetically encoded calcium indicators
- MDC have assayed electrical activity in control and HD iPSC-derived neurons by:

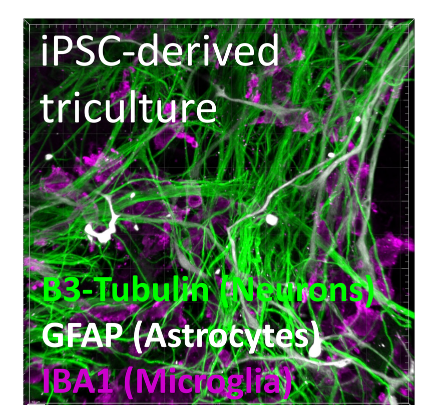
1. Loading of the calcium indicator Fluo4-AM and imaging on a Zeiss LSM880
2. Lentiviral transfection of a genetically encoded calcium indicator driven by a neuron-specific synapsin promoter and imaging with an Incucyte SX5

1. Fluo4 calcium imaging
2. Genetically encoded calcium imaging



Conclusions

- The use of iPSC-derived CNS cell models gives an opportunity to model disease and test the effect of drugs *in vitro*
- Methods such as immunocytochemistry, live-cell imaging and calcium imaging can provide ways to probe the effects of disease on morphology and neuronal function, and the subsequent effect of therapeutics in order to facilitate drug discovery
- CNS disease models can be simple, such as monocultures of neurons engineered to contain a genetic mutation, or more complex systems such as co-cultures or tri-cultures of multiple cell types



- The data on this poster has utilised a neuronal model of HD as an example, but the methods outlined could also apply to the study of other CNS diseases such as Parkinson's or Alzheimer's
- These types of models and *in vitro* techniques are invaluable to drug discovery as they further the understanding of disease phenotypes and provide a way to test therapeutics in a human system early on in the drug development pipeline

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