Project Details	
Project Code	MRCNMH25Ex Brown
Title	Enhancing stem cell-derived motor neuron function as a therapeutic approach in Amyotrophic Lateral Sclerosis
Research Theme	Neuroscience & Mental Health
Summary	Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, for which there is currently no effective treatment. In this project, you will use human motor neurons derived from patient stem cells, which develop features of neurodegeneration. Using electrophysiological approaches, you will examine the impact of ALS genotypes on the electrical and synaptic properties of these neurons. Further, you will attempt to rescue these electrical deficits by modulating specific genes identified from transcriptomic data and explore the underlying molecular pathways. The key aim is to identify novel drug targets for the treatment of ALS.
Description	Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative condition characterized by the loss of motor neurons (MNs). ALS patients experience progressive immobility, paralysis, and ultimately die from respiratory muscle failure within 3-5 years post-diagnosis. Understanding the molecular events leading to MN degeneration in ALS is crucial for developing therapies to halt or reverse the degeneration. Dysregulation of RNA processing, particularly alternative splicing, has emerged as a key molecular phenotype in most ALS cases. However, the role of splicing defects in MN homeostasis and degeneration in ALS remains poorly understood. Our proposal aims to investigate how RNA splicing defects cause MN dysfunction using MNs derived from human induced pluripotent stem cells (iPSCs). In particular, the student will use electrophysiological approaches to understand the impact of RNA splicing deficits on intrinsic excitability and synaptic function. These findings will deepen our understanding of MN death and dysfunction mechanisms in ALS and highlight the role of RNA processing in neurodegeneration, potentially unveiling new therapeutic avenues. RNA Splicing Defects in ALS Approximately 85% of ALS cases are sporadic (sALS) with no family history, while 15% are familial (fALS), often linked to specific genetic mutations. A key biochemical hallmark of almost all ALS patients is the cytoplasmic mislocalization and aggregation of TDP43, an RNA-binding protein that normally regulates RNA metabolism, including splicing, in the nucleus. This mislocalization is associated with the inclusion of cryptic microexons (CE) in genes such as STMN2 and UNC13A, leading to truncated, non-functional versions of proteins critical for synaptic vesicle recycling and axonal integrity. Human iPSCs Models of ALS We will use patient-derived iPSCs to generate human MNs, which exhibit disease-associated phenotypes and dysregulated pathways induced by underlying mutations. Using human iPSC-derived neurons is particularly relevant for ALS research as CEs identified in ALS/FTD cases are poorly conserved beyond primates. TDP43 mislocalization, sufficient to drive neuronal dysfunction and death, will be modelled using our innovative system that triggers cytoplasmic mislocalization of endogenous, non-mutated TDP43 in human iPSC-derived MNs, mirroring sporadic ALS

	biochemistry without external stressors. We will also use MNs differentiated from fALS TDP43 iPSCs and their isogenic corrected controls to investigate splicing defects. Transcriptomic and pathway analysis of our human MNs that display TDP43 mislocalization revealed downregulation of genes involved in cytoskeleton integrity, synapse formation and neurotransmission. Thirteen of these genes were also found to display the same splicing aberrations in ALS/FTD patient cortical neuronal nuclei. Neuronal hyperactivity followed by hypo-activation is commonly observed in ALS MNs prior to death. Hence, we hypothesize that rectifying neuronal firing by targeted genetic manipulation of candidate genes will enhance MN survival and function. In this project, the student will evaluate how these 13 target genes contribute to motor neuron electrophysiological function. Objectives: 1. Characterize functional consequences of perturbing candidate genes in human MNs. We will inhibit expression of candidate genes using CRISPR-mediated knockout in MNs after triggering TDP43 mislocalization. In parallel, we will also express the full-length protein to assess phenotypic rescue. After gene perturbation, we will assess MN synaptic transmission and neuronal activity using multi-electrode arrays and patch-clamp electrophysiology. The genetic manipulations will be carried out under the supervision of Bhinge in collaboration with the post-doc funded on the MRC project grant, whilst the electrophysiological assays will be performed in Brown's lab, working with another post-doc with long-standing electrophysiological expertise. 2. Test combinations of the top candidates identified in Objective 1 in rescuing disease phenotypes. It is likely that dysregulation of genes in different pathways important for neuronal function contribute synergistically to the observed MN dysfunction in ALS. Hence, we will modulate our candidate genes in combinations to evaluate whether targeting multiple points of failure within the neuronal gene network can better rescue disease phenotypes. We will select a subset of candidate genes that have shown significant improvements in at least one electrophysiological phenotype assayed as described above. Genes implicated in different pathways will be targeted (expressed or knocked down) together and phenotypes assessed as above. The student will play a key role in determining the gene candidates to combine and in selecting the appropriate functional assays. 3. Explore mechanism of action of the identified candidates. We will investigate localization and expression of the expressed protein in iPSC-derived MNs and ALS post-mortem tissue using immunohistochemistry. Further biochemical analysis on the stoichiometry of protein subunits and their stability will reveal how splicing changes might contribute to MN functional deficits. This work will be informed by results obtained from the previous objectives and will be influenced by Wilkinson's expertise in biochemical pathway analysis. With guidance from the supervisory team, the direction of the mechanistic studies will be determined by the student.

Supervisory Team	
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