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Thesis abstract

Modelling neuronal excitability changes in ALS using iPSC-derived motor neurons and astrocytes

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Amyotrophic Lateral Sclerosis (ALS) is the most severe form of Motor Neurone Disease. This fatal neurodegenerative disease causes the deterioration of the motor system leading to progressive paralysis. One of the earliest clinical observations in ALS patients is hyperexcitability of motor neurons in the motor cortex and spinal cord, prior to hypoexcitability and deterioration of motor function. The overarching objective of this thesis was to use induced pluripotent stem cells (iPSCs) from ALS patients to investigate electrophysiological changes to motor neurons, which may impact ALS progression. Firstly, the differentiation conditions of iPSC-derived motor neurons were optimised to improve the yield of electrophysiologically active motor neurons. Whole-cell patch clamping revealed that neuronal Na^+ and K^+ currents increased by more than 3-fold with the optimised culture conditions. Moreover, the proportion of repetitively firing neurons increased from less than 5% to approximately 75%. Secondly, using these improved culture conditions, the electrophysiological properties of *CCNF*^{S621G} motor neurons were compared to CRISPR/Cas 9 generated isogenic control cell lines. The *CCNF*^{S621G} motor neurons showed 3-fold increase in repetitively firing neurons compared to control motor neurons. This was further accompanied with a significant increase in Na^+ and K^+ currents. Together this suggests that the *CCNF*^{S621G} mutation alters the electrophysiological properties of motor neurons leading to neuronal hyperexcitability. Finally, iPSC-derived motor neuron and astrocyte co-cultures were used to investigate the effect of ALS astrocytes on motor neuron excitability. ALS-derived astrocytes caused a loss of neuronal firing in both ALS and control motor neurons. Moreover, Na^+ and K^+ currents were reduced by up to 55% and 30%, respectively. Together these findings showed that the addition of ALS astrocytes induced hypoexcitability in ALS, as well as control motor neurons. This suggests that ALS astrocytes could be involved in the transition from motor neuron hyperexcitability to hypoexcitability.

Overall the work presented in this thesis showed that the *CCNF*^{S621G} mutation causes hyperexcitability in iPSC-derived motor neurons. However, this hyperexcitability phenotype was lost in the presence of ALS astrocytes. Thus, this work highlights that the cellular crosstalk between motor neurons and astrocytes plays a significant role in altering intrinsic neuronal excitability, which could impact ALS progression.

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