PHYSIOLOGICALLY RELEVANT MEDIA UNMASKS SEVERE MITOCHONDRIAL DYSFUNCTION IN A PRECISION REPROGRAMMED IPSC-DERIVED MODEL OF HUNTINGTON'S DISEASE

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Abstract

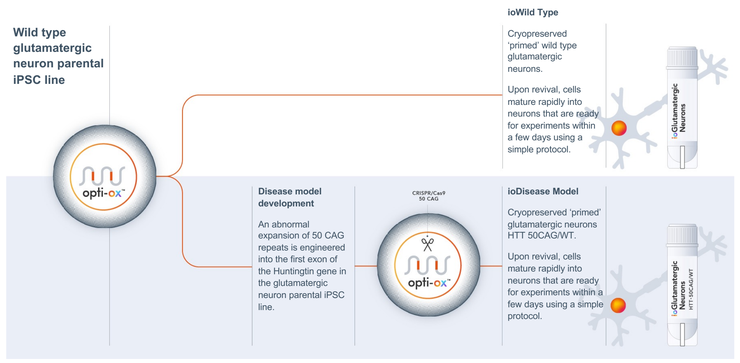
Huntington's disease (HD) is a devastating disease characterised by degeneration of the medium spiny neurons (MSNs) in the striatum. HD patients suffer from uncontrollable movements as well as severe mental problems and currently no disease modifying treatments are available. HD is an autosomal dominant disorder caused by a CAG repeat expansion encoding an elongated polyglutamine (PolyQ) stretch in the Huntingtin (HTT) protein. Although the precise pathogenic mechanisms remain poorly understood, the mutant aggregation-prone HTT protein has been reported to affect various cellular processes, including the biogenesis, fission, transport and respiration of mitochondria. HTT is ubiquitously expressed in the brain and, albeit MSNs are the most susceptible neurons to the toxic effects of mutant HTT protein, other neuronal subtypes such as the cortical glutamatergic neurons are affected during later disease stages.

We developed a novel iPSC-derived HD model based on our opti-ox™ deterministically programmed ioGlutamatergic Neurons. These ioGlutamatergic Neurons HTT 50CAG/WT contain a genetically engineered heterozygous 50 CAG repeat expansion in exon 1 of huntingtin. To investigate mitochondrial function in our HD model, cells were cultured in Neurobasal medium for 11 days and analysed with a Seahorse assay. The HD model showed a significant but modest reduction in basal and ATP-linked respiration relative to the wild type isogenic control. Unexpectedly, at day 25 oxygen consumption rates (OCR) in both genotypes were highly similar as neurons switched from mitochondrial respiration to glycolysis. Interestingly, culturing cells in a more physiologically relevant medium supported mitochondrial respiration at day 25 and unmasked a dramatic and significant mitochondrial dysfunction in the HD model. As neuronal firing is energy demanding, we assessed by high-density microelectrode array (HD-MEA) recordings whether mitochondrial dysfunction in the HD model affects neuronal activity relative to wild type cells. Culturing the cells for more than 30 days in a physiologically relevant medium significantly decreased the firing amplitude and rate as well as network activity in the HD model.

Overall, we have developed a scalable and consistent human HD model that recapitulates critical disease aspects and enables disease mechanistic and drug discovery studies.

1. Generation of a 50 CAG trinucleotide repeat expansion in the huntingtin gene

A heterozygous 50 CAG repeat expansion was introduced into the HTT gene of the wild type ioGlutamatergic Neurons cell line using CRISPR/Cas9 gene editing to generate a genetically matched disease model.

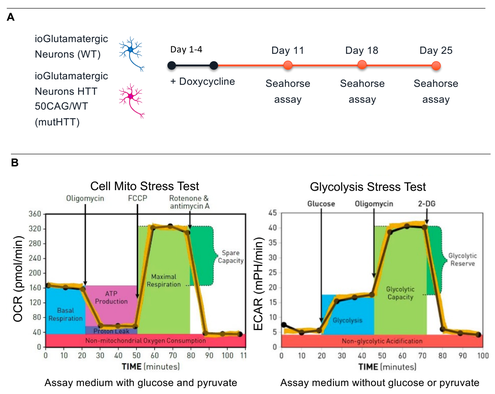


2. Assessment of mitochondrial function in Huntington's disease model cells

(A) ioGlutamatergic Neurons wild type (WT) and ioGlutamatergic Neurons HTT 50CAG/WT (mutHTT, HD model) were seeded at a density of 300K cells/cm² in Agilent Seahorse XF96 cell culture microplates and cultured according to the bit.bio user manual protocol in neurobasal media.

On days 11, 18 and 25 the cells were switched to Seahorse assay media 1 hour prior to analysis. The plates were imaged for cell counting after Seahorse assay to normalise OCR to the number of cells.

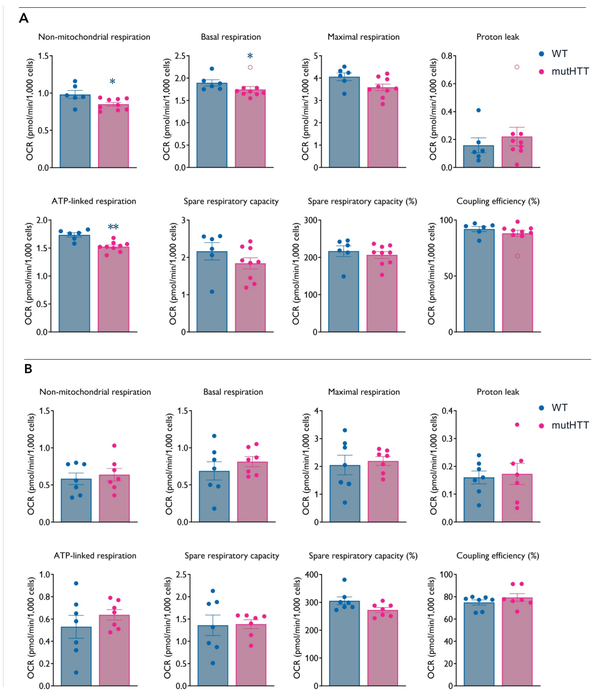
(B) Mitochondrial function was assessed using the Agilent Seahorse XF Analyzer, which measures the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in real time.



3. No major differences in mitochondrial respiration between wild type and HD model cells

(A) On day 11, non-mitochondrial, basal and ATP-linked respiration were slightly reduced in the HD model compared to the WT control.

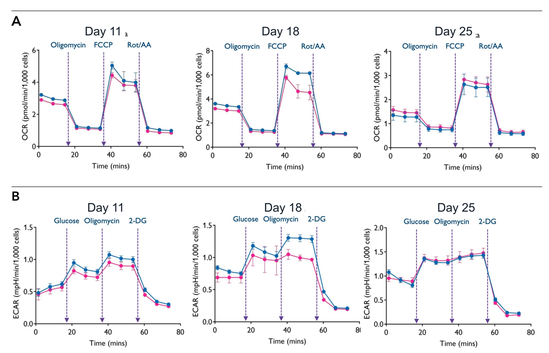
(B) On day 25, differences in mitochondrial respiration between the WT and HD model cells were no longer detected.



4. ioGlutamatergic Neurons switch from mitochondrial respiration to glycolysis

The graphs show that OCR reduced over time (A), and the glycolysis rate (ECAR) increased (B).

ioGlutamatergic Neurons switched to glycolysis over the time course of the experiment, therefore it was not possible to assess mitochondrial dysfunction in the HD model cells, masking a potential mitochondrial dysfunction phenotype

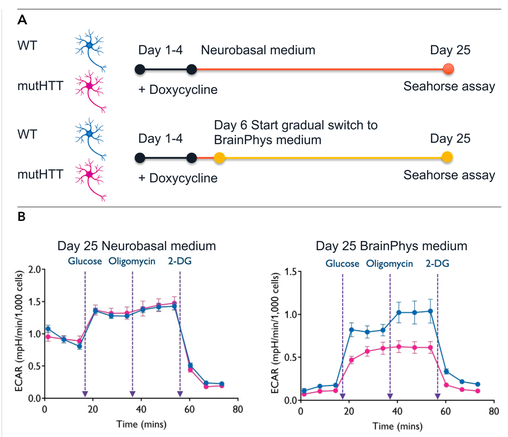


5. BrainPhys significantly lowers glycolysis in ioGlutamatergic Neurons

It has been shown that neurons can switch to glycolysis during maturation (Tourigny et al., 2019), and that BrainPhys medium can support mitochondrial respiration (Faria-Pereira et al., 2022).

(A) Overview of experiments to test the effect of BrainPhys medium on the glycolysis rate, compared to Neurobasal medium.

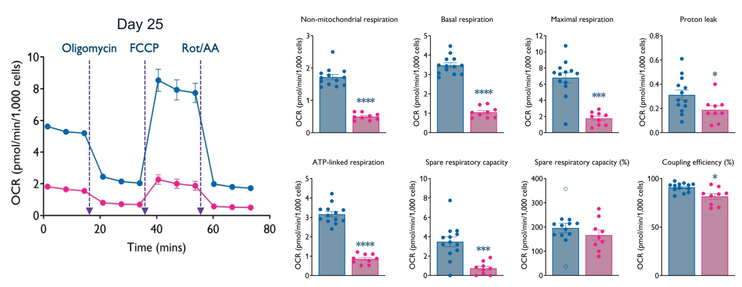
(B) When cultured in Neurobasal medium, the cells had a high basal level of glycolysis at day 25 (left). In contrast, when the cell culture medium was switched to BrainPhys, a low basal level of glycolysis was observed (right).



6. BrainPhys supports mitochondrial respiration and unmasks mitochondrial dysfunction in the HD model

Basal and maximal respiration were significantly increased in the WT cells compared to the HD model cells at day 25.

The HD model cells demonstrated mitochondrial dysfunction relative to the WT cells, but this was only observed when the cells were cultured BrainPhys medium.

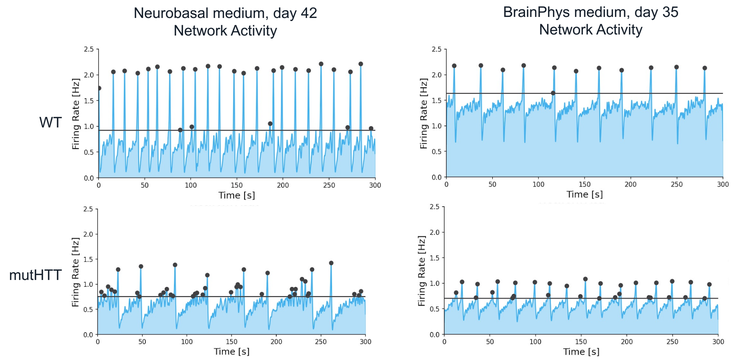


7. HD model shows reduced network activity compared to the genetically matched control

The network activity of the wild type control (WT) and HD model cells (mutHTT) cultured in Neurobasal or BrainPhys were compared using HD-MEA analysis (MaxTwo, MaxWell Biosystems).

Network activity was reduced in the HD model compared to the WT control in both Neurobasal and BrainPhys media.

In BrainPhys medium the synchronised and network activity develop earlier, leading to an observable phenotype at an earlier time point.



Summary & conclusions

Using CRISPR/Cas9 editing we successfully introduced a 50 CAG trinucleotide expansion in exon 1 of the HTT gene in an opti-ox enabled ioGlutamatergic Neurons cell line, creating a novel, genetically matched human iPSC-derived Huntington's disease model.

By culturing the HD model in a physiologically relevant medium that supported mitochondrial respiration, we unmasked a mitochondrial dysfunction phenotype. Interestingly, using MEA analysis a reduced network activity phenotype was observable in both media conditions

ioGlutamatergic Neurons HTT 50CAG/WT represent a robust and scalable human iPSC-derived Huntingdon's disease model with a physiologically relevant phenotype. Used with ioGlutamatergic Neurons, the genetically matched control, the HD model is suitable for translational research and drug discovery applications.