Human induced pluripotent stem cell-derived microglial systems of the hypothalamus for high-throughput target discovery in obesity

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Project outline

Background: Microglia (tissue-resident macrophages in the brain) can directly sense chronic nutrient excess in the brain, especially from saturated fatty acids, to which they respond by switching to a pro-inflammatory state (Valdearcos et al., 2014). Dampening this microglial response, particularly in the hypothalamus, in rodent models of diet-induced obesity results in protection against weight gain (Valdearcos et al., 2017), making microglia attractive targets for obesity therapeutics. Evidence in humans remain correlative, with post-mortem tissue sections and brain magnetic resonance imaging showing microgliosis to be correlated with BMI (Baufeld et al., 2016; Sewaybricker et al., 2023).

Hypothesis: Human iPSC-derived microglia reflect the behaviour of *in vivo* resident microglia, including phenotypes related to obesity such as inflammation in response to chronic nutrient excess. Co-culture with hypothalamic neurons will enhance these phenotypes by priming microglia towards a region-specific state. High-throughput screening in this model will bring forth new targets that when manipulated, will reduce inflammation, appetite and/or body weight.

Aims and description of work to be undertaken:

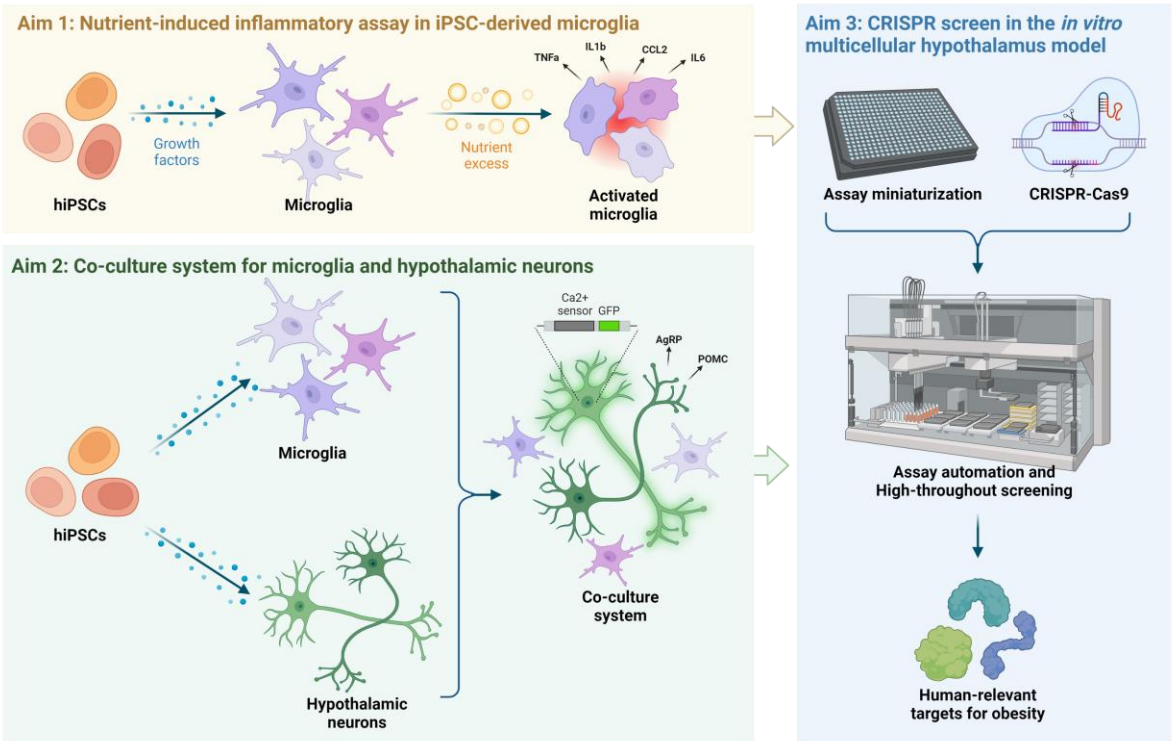
Aim 1: Development of nutrient-induced inflammatory assays in iPSC-derived microglia. We will first generate human microglia in culture from iPSCs following existing methodology established by Cowley's team (Washer et al., 2022) and test their response to excess nutrients, specifically saturated fatty acids, using readouts such as secretion of inflammatory cytokines e.g. TNF and MCP-1, expression of the surface phenotypic markers CD14 and CD16, and morphological changes from ramified to amoeboid. Readouts with the best assay windows will be identified in the process, and differentiation conditions will be adjusted and optimized to maximize the response in these assays.

Aim 2: Development of co-culture systems for iPSC-derived microglia and hypothalamic neurons. Hypothalamic neurons will be derived from iPSCs using optimized versions of published protocols (Rajamani et al., 2018), and will be combined with the iPSC-derived microglia from Aim 1 following co-culture optimization principles as demonstrated previously by Cowley's team (Vahsen et al., 2022). The resulting system will be tested for the readouts in Aim 1, as well as assessed using transcriptomics guided by region-specific RNA-seq datasets for microglia (Herb et al., 2023). Hypothalamic neuron

function will be assessed by measuring secretion of neurotransmitters like AgRP and POMC, by using genetic Ca^{2+} sensors engineered in the source iPSC line, by neuronal survival and morphology, and finally with our local research partner Metrion, by using multiple electrode array and patch clamp.

Aim 3. Genome-wide CRISPR screen in the *in vitro* multicellular hypothalamus model. The optimized differentiation protocols and readout assay(s) from Aims 1 and 2 will be brought forward for miniaturization in the 384-well or 1536-well format using automation platforms. Introduction of CRISPR/Cas9 constructs, whether by stable cell line generation or by ribonucleoprotein trans- or nucleofection, will then be optimized in the miniaturized format. Design of experiment principles for high-throughput screening will be applied to find the best parameters for a pilot screen targeting a set of ~100 genes implicated in appetite control and obesity (determined through the literature and *in silico* analytic methods combining genetics and transcriptomics of public datasets). This will then be expanded to perform the first whole-genome screen in human microglia in the context of obesity.

Expected outcomes: The project will find novel genes that when manipulated, reduces inflammation and improves hypothalamic neuron function. *In vivo*, these genes have the potential to modulate appetite and body weight, which can be further investigated. The human iPSC-derived models developed here can be used for future studies including testing tool compounds for studying obesity. This collaborative project will offer excellent training opportunities in the application of cell culture techniques, stem cell biology, immunobiology, neurobiology, automation and drug discovery.



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Dr Sally Cowley

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Dr Kevin Gonzales

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