Development of iPSC-derived Alzheimer Disease (AD) platforms for phenotypic screening

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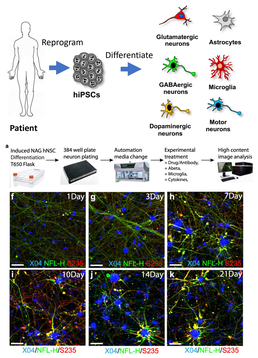
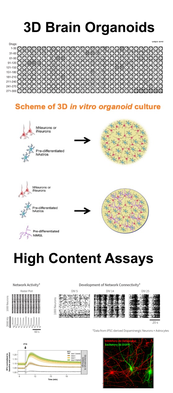
Abstract

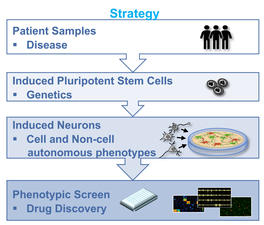
Here we describe the development of iPSC-derived platforms of AD that can be used to screen for preclinical targets and drug candidates. The foundation of these platforms utilizes a proprietary method to generate pure populations of either NGN2-excitatory or ASCL1/DLX2-inhibitory neurons. We developed a neuron/astrocyte co-culture platform to uncover *APOE4*-specific electrophysiological phenotypes in which *APOE4* is the greatest genetic risk factor of sporadic AD. This platform can be complemented with isogenic iPSC-derived astrocytes and microglia to interrogate both cell autonomous and non-cell autonomous mechanisms for genetic risk factors that either confer increased or decreased AD risk. We also report the development of an iPSC-based transgenic tau overexpression platform to investigate how different tau isoforms promote prion-like tau spreading and be used to screen for compounds to mitigate tau spreading. Tau overexpression causes neuron-neuron tau transmission and leads to the accumulation of key neuropathological tau biomarkers. Taken together, these innovative phenotypic assays can be translated to high-throughput drug screening efforts for AD.

Induced Pluripotent Stem Cells Facilitate Translational Disease Modeling for Drug Discovery

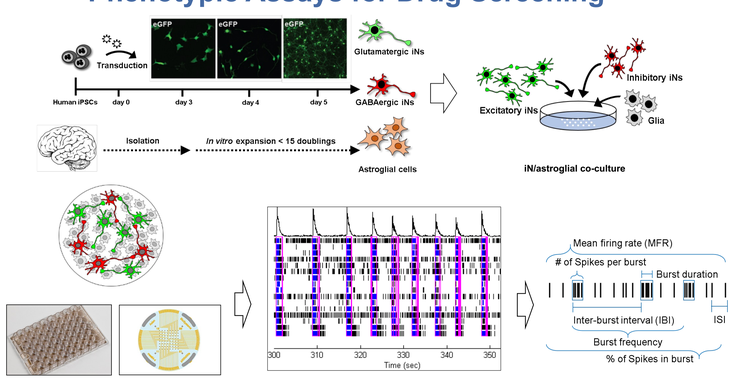


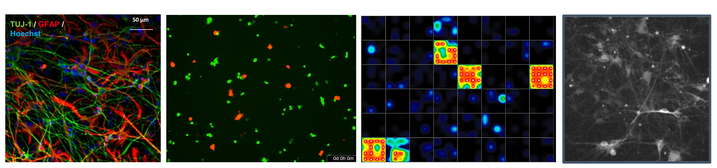
2D "Brain in a Dish" (Adapted from Bassil et al., 2021)



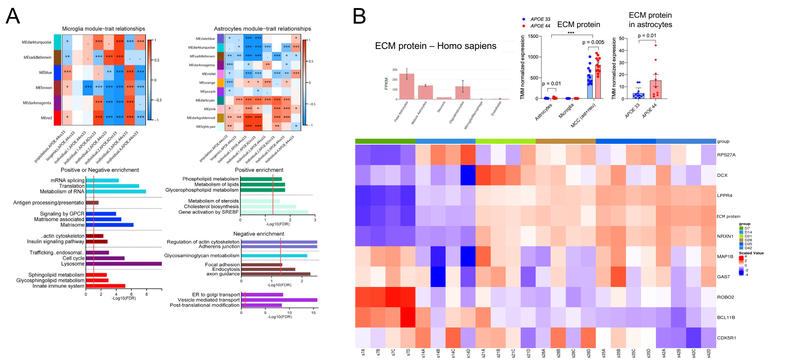


NeuCyte SynFire® Platform Enables Development of Phenotypic Assays for Drug Screening



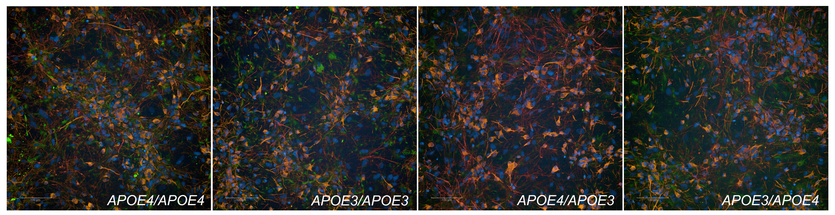


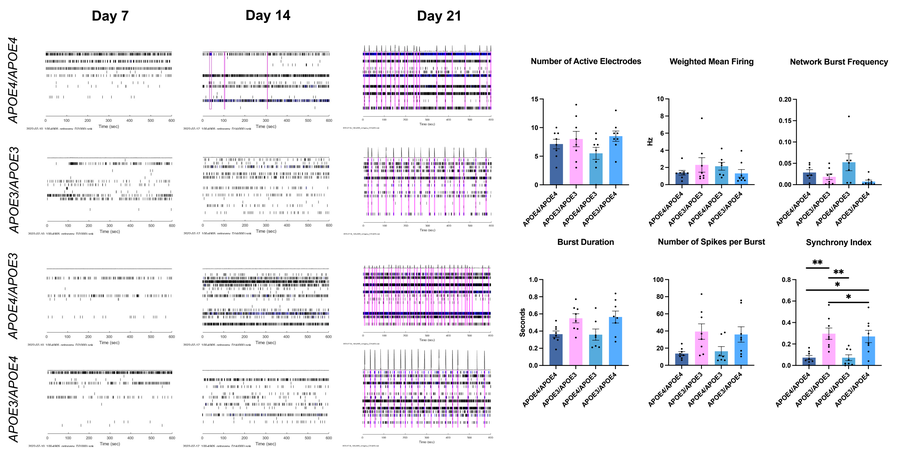
APOE Isogenic iPSCs Facilitate Human-specific Phenotypic Discovery



(A) Cell autonomous phenotypes in iPSC-derived astrocytes and microglia due to *APOE4* (Adapted from TCW et al., 2022). **(B)** Non-cell autonomous *APOE4-specific* increase in matrisome signaling i.e., ECM protein upregulation are captured in astrocytes from neuronal/astrocyte co-cultures and mimicked in induced excitatory/inhibitory neuron/primary astrocyte co-cultures

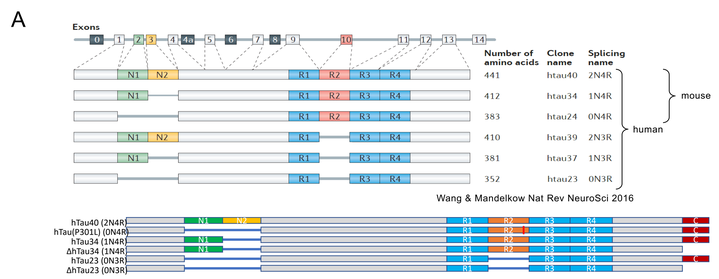
Development of an Electrophysiological MEA Phenotypic Assay for *APOE4*

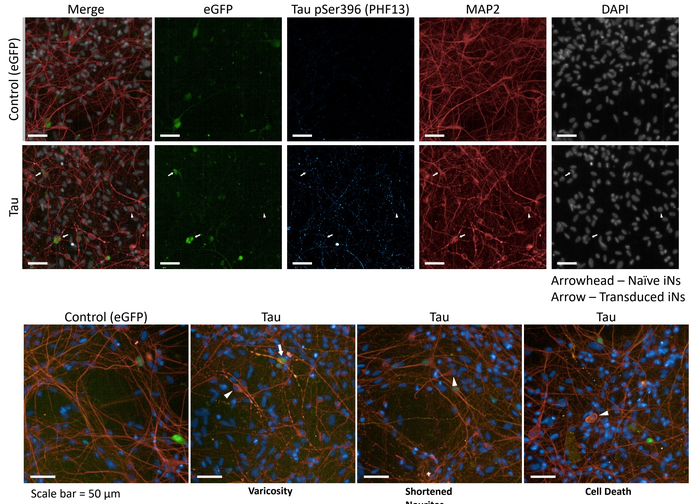


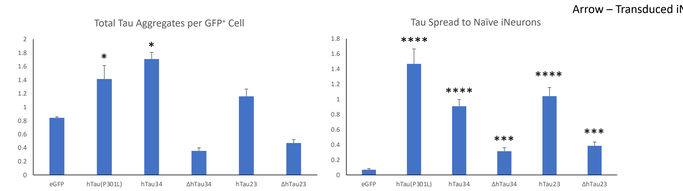


(A) Representative immunocytochemical images of induced excitatory and inhibitory *APOE4* and APOE3 neurons cultured on primary astrocytes (DPP14). **(B)** Representative raster plots demonstrating the normal development of mature neuronal networks over 21 days. **(C)** *APOE4* excitatory neurons exhibit impaired network synchrony.

Development of an iPSC-based Tau Pathological Screening Assay for Drug Discovery







(A) Human iPSC neurons express human tau isoforms. Due to tau isoforms differences between mouse and humans, iPSC models facilitate interrogation of relevant and translational tau-based mechanisms in which examination of tau pathological mechanisms will include the full complement of spliced tau isoforms. **(B)** Representative images showing tau transmission between neurons. Scale bar = 50 µm. **(C)** Tau overexpression leads to axonal varicosities, decreased neurite length and cell death. Blue = DAPI, Green= eGFP, Red = Tau pSer202 pThr205 (AT8), Far Red = MAP2, scale bar = 50 µm. **(D)** Quantification of tau aggregates and tau transmission by different tau isoforms in wild-type iNeurons.

Future Directions

- Incorporate iPSC-derived Astrocytes and Microglia into Co-cultures for investigating Neuroinflammatory mechanisms
- Develop 3D organoid/assembloid screening assays
- Translate Phenotypic assays to Drug Screening and Drug Discovery
- Examine APOE4/tau/GABA tripartite relationship