Development of iPSC-derived Astrocyte and Neuronal Co-culture Models to study Neuronal Functionality using Multielectrode Arrays

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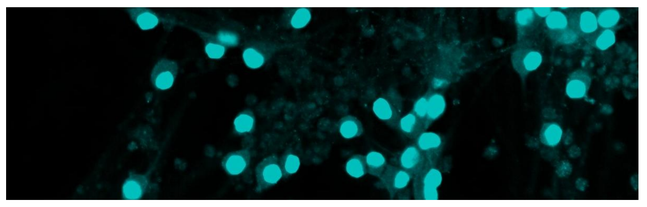

Abstract

Astrocytes have an important role in maintaining homeostatic conditions in the brain. Astrocytes are involved in many processes such as clearing excess neurotransmitters, stabilizing and regulating the blood-brain barrier, and regulating axonal growth. Induced pluripotent stem cell (iPSC) technology has made it possible to work with human astrocytes and neurons. However, many iPSC-derived cell models consist of pure neuronal cultures or rely on astrocytes formation as a byproduct during the neural differentiation, which in turn does not allow for cultures with defined ratios of neurons and astrocytes. As such there is a high need for defined co-culture models, which incorporate astrocytes and neurons for improved modelling of neurodegenerative diseases.

Here, we report on the use of an efficient protocol to generate human iPSC-derived astrocytes and how they can be implemented in neural co-culture systems to improve the study of functional cell responses using the multielectrode array (MEA) system.

Our results show that co-cultures with neurons and astrocytes, compared to neuronal mono-cultures, have improved firing rate, spike amplitude, and activity in the MEA. This strongly shows the importance of including astrocytes in neural modelling when studying electrophysiological functionality.

hiPSC-derived Astrocytes and Neurons co-cultured on MEA

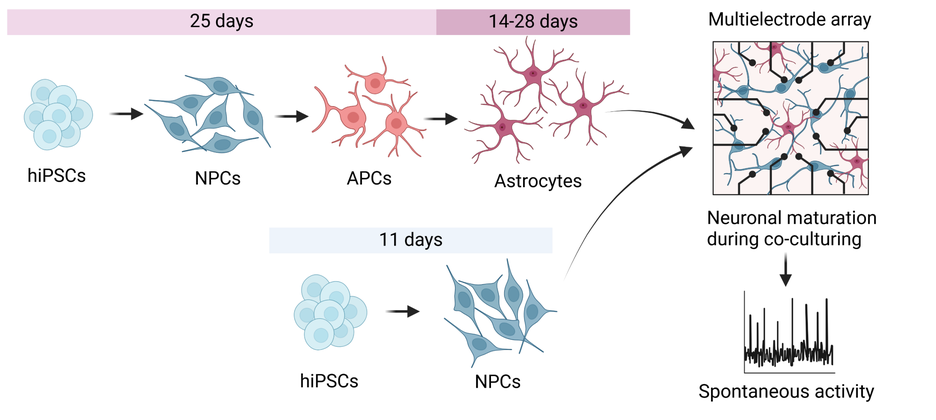

Figure 1. Differentiation of hiPSC-derived astrocytes and sensory neurons in a co-culture model. hiPSCs were differentiated to astrocytes or sensory neurons and co-cultured on multielectrode arrays on the MaxTwo High Density MEA system to evaluate neuronal functionality. Spontaneous activity was detectable after 14 days of co-culturing.

Expression of Cell Type Specific Markers

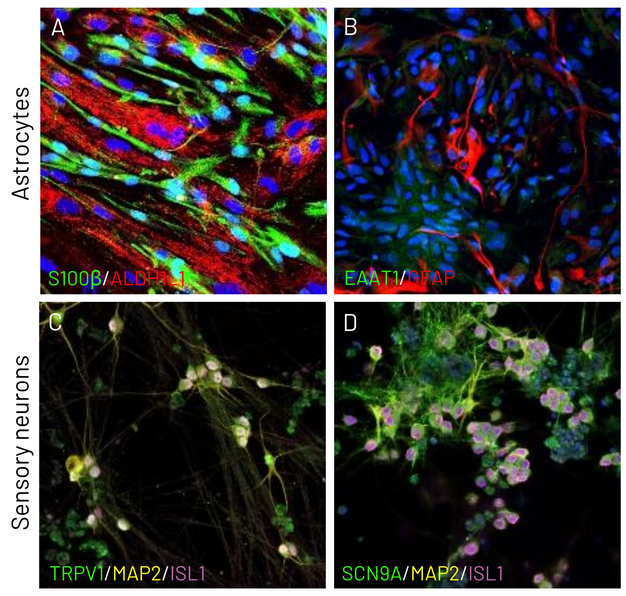

Figure 2. Representative immunofluorescent images of hiPSC-derived astrocytes (top panel) and hiPSC-derived sensory neurons (bottom panel) (20x, 2x crop). (A) Mono-culture of astrocytes stained for the astrocyte specific markers S100β and ALDH1L1, and **(B)** EAAT1 together with GFAP. **(C, D)** Mono-cultures of neurons stained for the sensory neuron specific markers TRPV1, ISL1, and SCN9A together with MAP2.

References

1. Plumbly *et al.* Derivation of nociceptive sensory neurons from hiPSCs with early patterning and temporally controlled *NEUROG2* overexpression. Cell Rep Methods. 2022.
2. Stacey *et al.* Plate-Based Phenotypic Screening for Pain Using Human iPSC-Derived Sensory Neurons. SLAS Discov. 2018.
3. Stoklund Dittlau *et al.* Generation of Human Induced Pluripotent Stem Cell (hiPSC)-Derived Astrocytes for Amyotrophic Lateral Sclerosis and Other Neurodegenerative Disease Studies. Bio-protocol. 2024.

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Indicative Stain of mono- and co-cultured Sensory Neurons

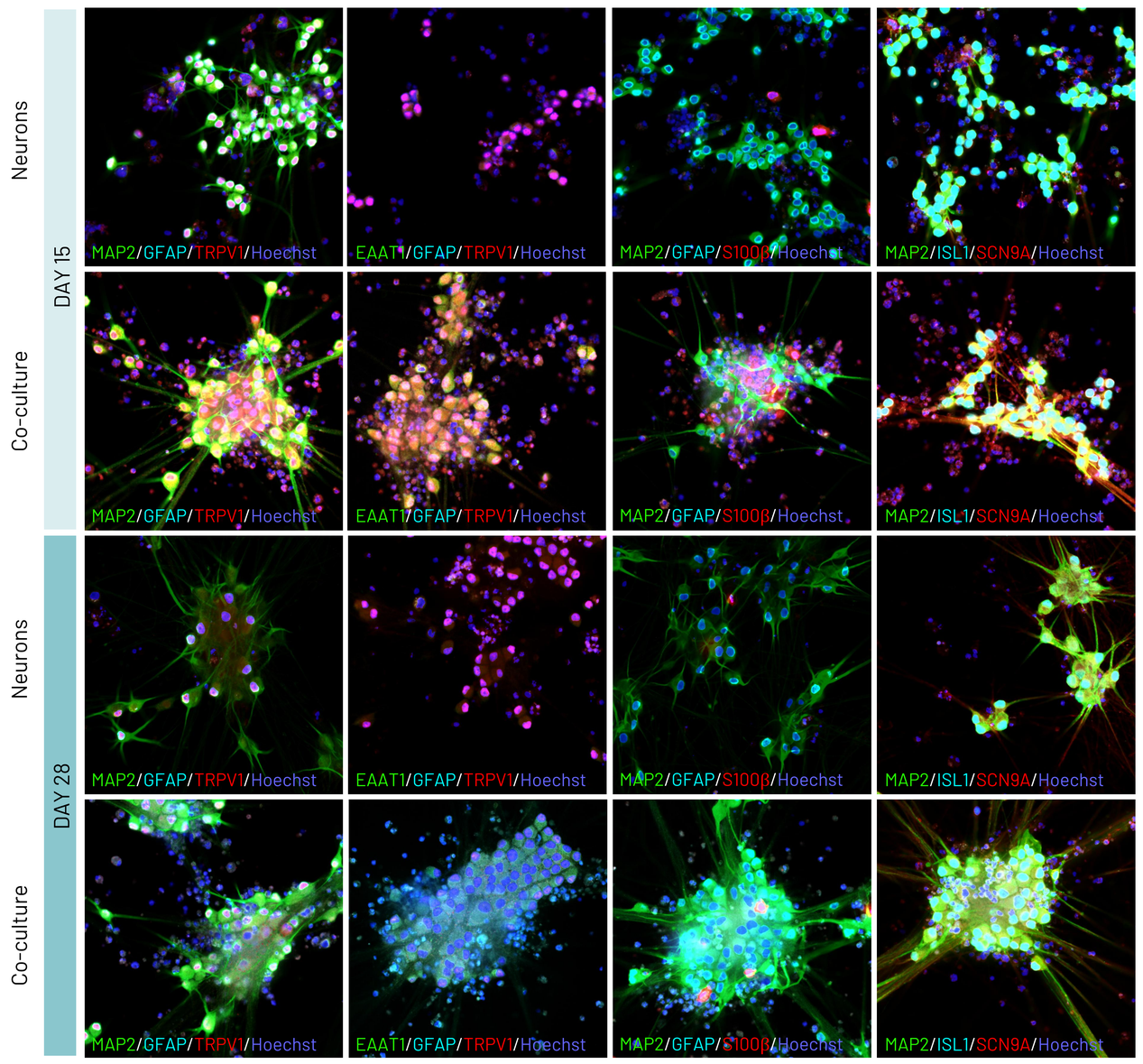

Figure 3. Representative immunofluorescent images of hiPSC-derived sensory neurons in mono- and co-culture with hiPSC-derived astrocytes (20x, 2x crop). The co-cultured neurons show higher expression of the neuronal markers, TRPV1, and SCN9A compared to mono-cultures. When neurons are cultured with astrocytes they form clusters and stronger connections.

Astrocytes enhance Neuronal Activity

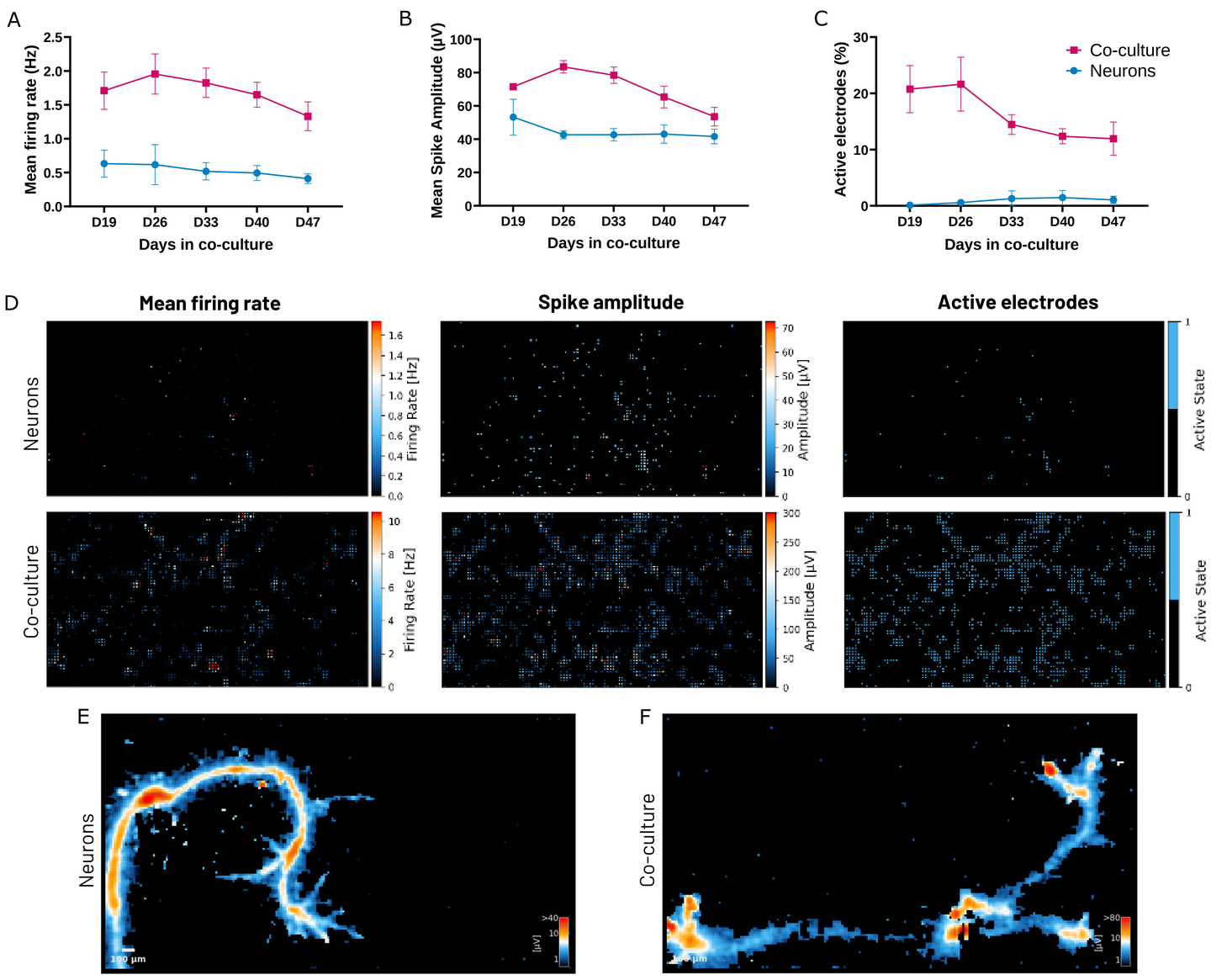

Figure 3: Neurons in co-culture with astrocytes show higher mean firing rate and spike amplitude. (A) Mean firing rate (Hz) over time for neurons in mono-culture (blue) and in co-culture with astrocytes (pink). **(B)** Neurons in co-culture show a larger spike amplitude and **(C)** a larger number (%) of active electrodes. **(D)** Representative heat maps of the mean firing rate, spike amplitude, and active number of electrode for neurons in mono-culture and co-culture obtained at day 26**. (E)** The axonal footprint in mono-culture and **(F)** co-culture obtained from the MaxTwo MEA system.

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