Application Note

Ion Channels:
Na_V, K_V, $GABA_A$, glycine

Cells:
hiPSC-derived neurons (EBiSC-NEUR1)

Tools:
SyncroPatch 384

The impact of astrocytes on the maturation of hiPSC-derived neurons

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Summary

Human induced pluripotent stem cells (hiPSCs) are becoming increasingly used in biomedical research for disease modelling and drug discovery. The use of stem cells for research reduces the need for animals, as well as having the added advantages of being human in origin and available in relatively high abundance. One of the challenges in the use of hiPSCs is their proposed immaturity compared with native cells, potentially limiting their usefulness as a model system. In order to address this limitation, culture conditions including media supplements, or co-culture conditions are being investigated. Indeed, in a recent study, the co-culture of hiPSC-derived atrial cardiomyocytes with fibroblasts induced functional maturity of atrial (but not ventricular) cardiomyocytes [1].

Stem cell-derived neurons have become important tools in neuroscience research and a number of hiPSC-derived neuronal types are currently available on the market. Culture conditions are critical for maturation of hiPSC-derived neurons and it was discovered early on that commonly used culture media, e.g. DMEM-basal, Neurobasal or serum, while promoting neuronal survival, could actually impair neurophysiological functions [2]. In order to promote maturation of neurons, they can be co-cultured with astrocytes [3,4,5].

In collaboration with the Fraunhofer Institute for Biomedical Engineering (IBMT) we explored co-culture of hiPSC-derived neurons (EBiSC-NEUR1) [6] and astrocytes (ScienCell, #1800) by comparing the electrophysiological properties of the neurons using the SyncroPatch 384.

Results

hiPSC-derived neurons were either cultured alone (N) or co-cultured with astrocytes (N+A) and the success rate for cell capture and presence of Na_V and K_V currents was compared (Figure 1A). In addition, cell size, current amplitude of K_V and Na_V currents, and V_{half} of activation and inactivation of Na_V currents were measured and compared (Figure 1; Table 1).

	Cm (pF)	Na_V peak (nA)	Na V_{half} (act; mV)	Na V_{half} (inact; mV)	K_V peak (nA)
N	6.2 ± 0.5 (37)	-1.0 ± 0.2 (37)	-17 (12)	-56.5 ± 1.5 (14)	0.9 ± 0.1 (56)
N+A	18.4 ± 2.9 (91)***	-3.3 ± 2.9 (91)***	-22 (48)	-58.9 ± 0.9 (65)	1.5 ± 0.1 (81)***

Table 1: Cell capacitance and current amplitudes of Na_V and K_V were significantly larger in neurons co-cultured with astrocytes. ***$P<0.001$.

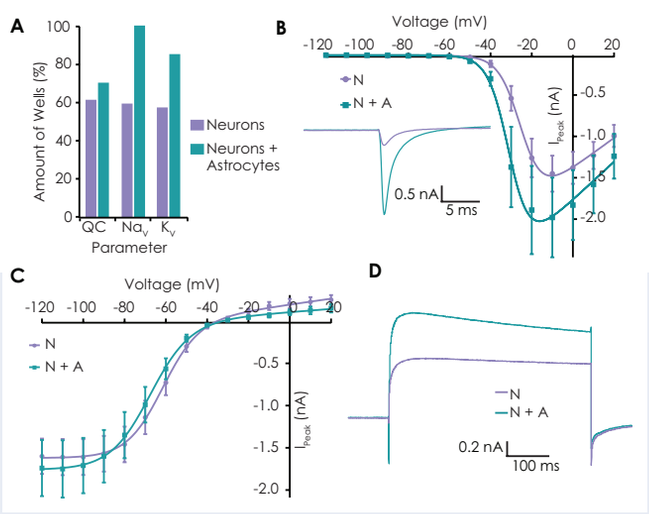

Figure 1: A *Bar graph of success rates for cell sealing (QC), and presence of Na_V and K_V current in different culture conditions.* **B** *Activation IV curves for hiPSC neurons in different culture conditions, with example traces at -10 mV.* **C** *Inactivation IV curve in different culture conditions.* **D** *Example traces of K_V currents in different culture conditions.*

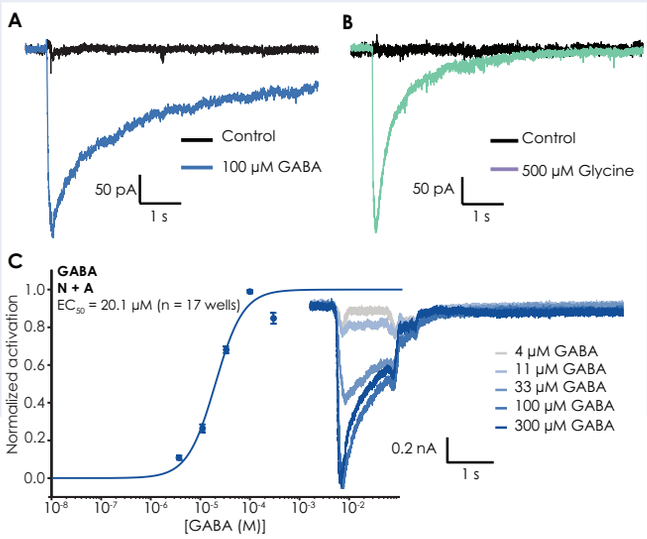

Figure 2: A *GABA responses to a single application of 100 µM GABA were recorded.* **B** *Glycine responses to 500 µM glycine were also recorded in some neurons.* **C** *GABA responses were concentration dependent and desensitization was observed at the highest concentration. The* EC_{50} *was 20.1 µM (n = 17 wells).*

Although success rates for cell capture were similar for the 2 culture conditions (60 - 70%), Na_V currents were detected in 100% of N+A, compared with 60% of N, with a similar result for K_V currents. In addition, Na_V current amplitudes were significantly larger in N+A *vs* N ($P<0.001$, unpaired Student's t test), presumably due to an observed increase in cell capacitance (Table 1). The V_{half} of activation and inactivation of Na_V currents, however, were similar between the two conditions, indicating that the Na_V subtype is unchanged. K_V-mediated currents were also significantly larger in N+A *vs* N.

In preliminary experiments looking at different ligand-gated ion channels, we could identify a GABA-mediated response and glycine-mediated responses in both N+A and N culture conditions (Figure 2). There appeared to be little difference in the number of cells, nor the current amplitudes of GABA and glycine responses recorded in the two conditions on the small number of cells tested so far. A GABA concentration response curve revealed an EC_{50} for GABA of 20.1 µM (n = 17 wells). Small responses to acetylcholine, Bz-ATP and glutamate were detected in a proportion of N and N+A neurons (data not shown).

In summary, hiPSC-neurons cultured under different conditions, either with or without co-culture with astrocytes, could be captured with excellent success rates (70%) and differences could be observed in cell parameters and ion channel current amplitudes. Further tests are required to determine effects on neuron excitability, by recording resting membrane potential and action potentials in current clamp mode.

References

1. Brown, G.E., *et al.*, 2024. Sci. Adv. 10: eadg1222.

2. Bardy C., *et al.*, 2015. PNAS. 112 (20) E2725-E2734.

3. Hedegaard, A., *et al.*, 2020. Stem Cell Reports. 15: 38–51.

4. Shih, P-Y., *et al.*, 2021. Stem Cell Research. 54: 102386.

5. Supakul, S., *et al.*, 2024. Inflammation and Degradation. 44: 8.

6. Wihan, J., *et al.* 2023. J Vis Exp. 2023 Mar 17:(193).

Methods

Cells

Cultured hiPSC-derived neurons (EBiSC, EBiSC-NEUR1) cultured alone and co-cultured with astrocytes (ScienCell, #1800) were kindly provided by Fraunhofer IBMT.

Electrophysiology measurements

Cells were harvested according to Nanion's cell harvesting protocol for neurons. Cells were resuspended in external recording solution and stored in the cooled CellHotel of the SyncroPatch 384, shaking at 15°C before use. Internal and external solution compositions are available upon request. Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384. Activation and inactivation IVs were recorded using a triple step protocol in PatchControl 384 and analysed using the IV analysis tool of DataControl 384. K_V currents were recorded using a single step protocol. Ligands GABA, glycine, acetylcholine, Bz-ATP and glutamate were applied using the 'LigandPuff' protocol, cells here held at -70 mV and briefly exposed to ligands (1 s). N and N+A run in parallel on the NPC-384 chips in each experiment.

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