Application Note

Channel: I_{Na}, I_K
Cells: hiPSC-derived neurons
Tools: Patchliner®

Peri.4U and Dopa.4U stem cell-derived neurons recorded on Nanion´s Patchliner®

The electrophysiology team at Nanion Technologies GmbH, Munich.
hiPSC neurons kindly provided by Axiogenesis, AG.

Summary

Human induced pluripotent stem cell-derived neurons (hiPSC-neurons) may provide a viable cellular model for studying the mechanisms underlying neurological diseases and drug development. Axiogenesis provides a number of hiPSC-neurons including dopaminergic neurons (Dopa.4U) and peripheral neurons (Peri.4U), amongst others. These neurons have been used on in-vitro systems such as multielectrode arrays (MEA), immunocytochemistry and calcium imaging. They are an interesting model for studying neurological diseases such as Parkinson's Disease, as well as for efficacy, drug discovery and toxicity studies.

In this study, the Patchliner was used to record from Dopa.4U and Peri.4U neurons in voltage and current clamp modes. The cell harvesting procedure was optimized (using papain) to ensure that the cells retained proximal dendrites and initial axon segments in order to maintain ion channel expression present in these regions. Due to their irregular shape (presence of processes), success rate (typically 20 - 50% for RSeal >200 MΩ) was lower than other cell types which have a smooth, round shape, e.g. standard cell lines. Voltage-gated Na (Na_V) and K (K_V) currents were recorded in both cell types. Action potentials (AP) were also recorded and block of the AP of Dopa.4U cells by lidocaine is shown.

Cell	RSeal (MΩ)	Na_V peak (pA)	K_V peak (pA)
Peri.4U	860 ± 101 (31)	586 ± 135 (8)	748 ± 191 (5)
Dopa.4U	852 ± 477 (6)	851 ± 300 (5)	555 138 (4)

Table 1: Seal resistance (RSeal) and peak amplitude values for Na_V and K_V recorded from Peri.4U and Dopa.4U cells recorded on the Patchliner®. Number of cells shown in brackets.

Results

Na_V and K_V currents of a Peri.4U cell with the corresponding IV plots from an average of 8 cells are shown in Figure 1. See Table 1 for average peak amplitudes. A Boltzmann equation was used to fit the Na_V IV curve and Vhalf = -19 mV. Figure 2 shows Na_V and K_V currents of a Dopa.4U cell with the corresponding IV plots from an average of 5 and 4 cells, respectively.

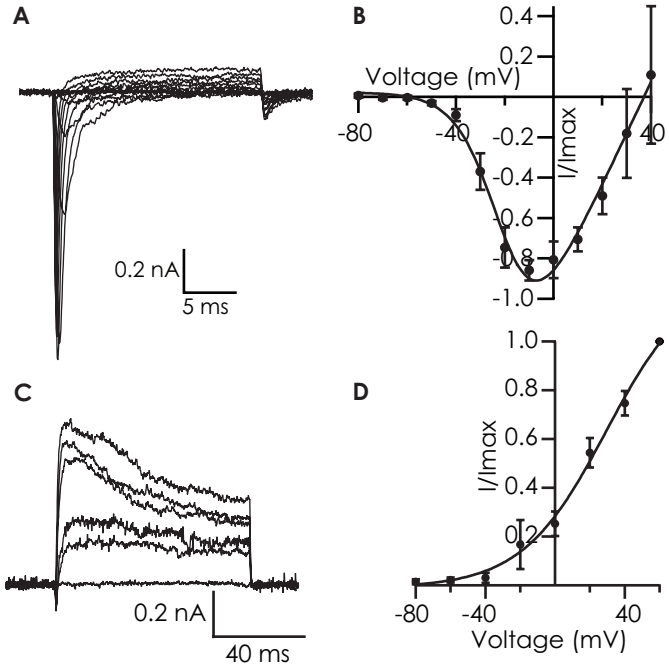

Figure 1: A Raw Na_V current traces from an exemplar Peri.4U neuron. **B** Corresponding IV plot for an average of 8 cells. **C** Raw K_V current traces from an exemplar Peri.4U neuron. **D** Corresponding IV plot for an average of 8 cells.

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A Boltzmann equation was used to fit the Na_V IV curve and Vhalf = -31mV.

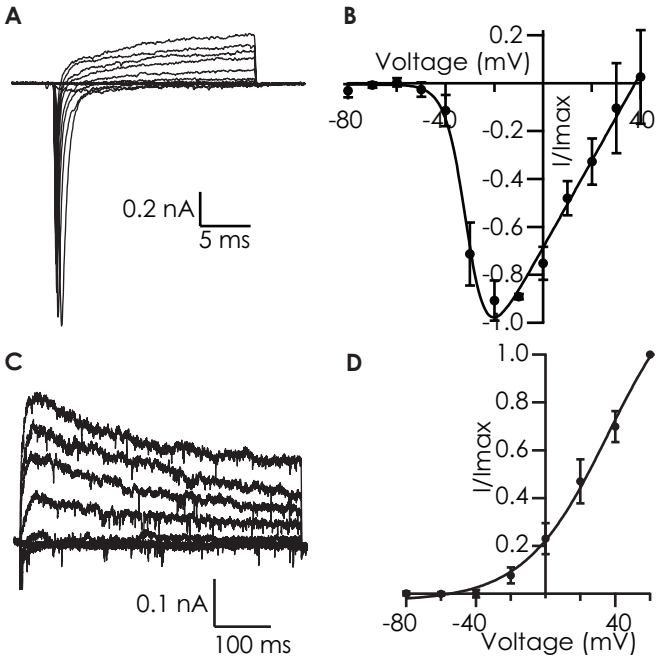

Figure 2: A Raw Na_V current traces from an exemplar Dopa.4U neuron. **B** Corresponding IV plot for an average of 5 cells. **C** Raw K_V current traces from an exemplar Dopa.4U neuron. **D** Corresponding IV plot for an average of 4 cells.

Figure 3 shows an AP elicited from a Dopa.4U cell and inhibition of the AP by lidocaine.

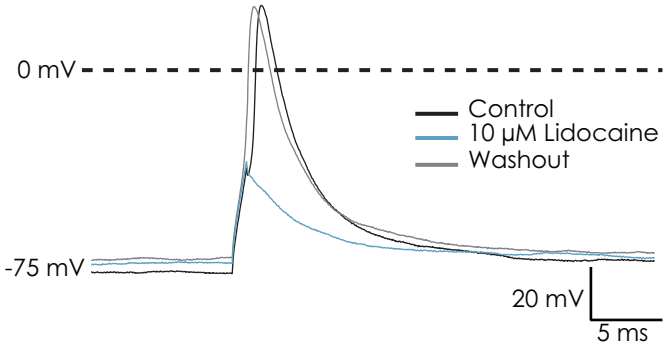

Figure 3: AP elicited from a Dopa.4U neuron using a 1 ms pulse to 80 pA in control (black) and in the presence of 10 µM lidocaine (blue). The AP could be recovered after washout of lidocaine (grey).

In summary, Peri.4U and Dopa.4U neurons from Axiogenesis can be used on the Patchliner® with a success rate of up to 50% (seal resistance > 200 MΩ). The harvesting protocol using papain was developed in an attempt to retain the cell architecture at the expense of capture rate. It should be noted, however, that the effect of detachment and loss of some neurite-located ion channels can change the profile of cell currents, resting membrane potential and responses to some neurotransmitters. Nevertheless, Na_V and K_V currents were recorded in voltage clamp mode and APs were elicited in current clamp mode. This provides the opportunity to combine a cellular neuronal model with higher throughput automated electrophysiology. In this way, such a cell model offers an alternative to primary neuronal cell cultures for studying neuronal toxicity, disease research and drug discovery.

Methods

Cells

Human iPS cell-derived neurons (Peri.4U™ and Dopa.4U™ neurons) from Axiogenesis AG were used.

Cell culture

Cells were received as frozen aliquots and were plated and cultured according to the manufacturer's instructions. Cells were harvested using papain.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner®. For Na_V currents, cells were stepped from a holding potential of -100 mV to -80 mV for 20 ms and then increasing in 10 mV increments with each sweep up to 60 mV. For K_V currents, cell were stepped from a holding potential of -80 mV to -60 mV for 200ms and then increasing in 20 mV increments with each sweep up to 60 mV. For current clamp recordings, cells were sealed and the whole cell configuration achieved in voltage-clamp mode, following which, cells were switched to current clamp mode. Current was injected to maintain a constant membrane potential of -75 mV (set individually for each cell) and action potentials were elicited using a 1 ms current pulse to the threshold required to elicit an action potential (set individually for each cell).

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