Application Note

Channel: I_{Na}, I_K, GABA
Cells: iPS cell-derived neurons
Tools: Patchliner®

Stem cell derived neurons recorded on Nanion´s Patchliner®

The electrophysiology team at Nanion Technologies GmbH, Munich.

Summary

Cellular Dynamics International is developing iCell® Neurons, human iPS cell-derived neurons. These neurons have been used on Nanion's Patchliner®, an automated patch clamp device for recording from up to 8 cells simultaneously. Cells were thawed and cultured as per the manufacturer's instructions. After plating, cells developed neuronal outgrowth within 24 hours and could be kept in culture for 2 weeks. An investigation into voltage- and ligand-gated ion channels expressed in these cells was undertaken using the Patchliner®. The first experiments are shown here and offer promising results for combining a cellular model for neurons and an increased throughput patch clamp device.

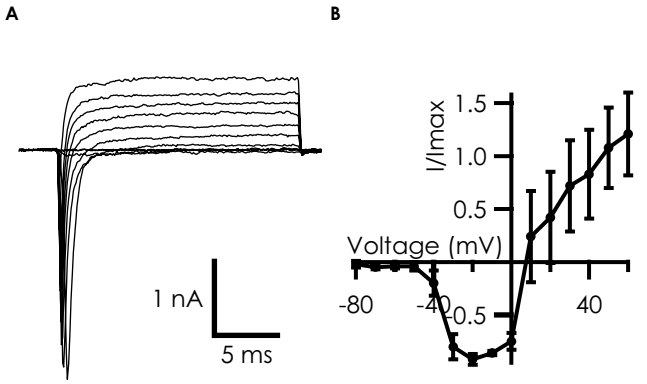

Figure 1:
A Voltage-gated Na^+ current recorded in iCell® Neurons. Current responses to a voltage step protocol. A large inward Na^+ current can be seen in this cell with a K^+ outward current present at positive voltages. **B** Normalised IV plot from an average of 4 cells.

Results

Voltage-gated Na^+ currents of an individual cell are shown in Figure 1. Not every cell showed expression of a Na^+ current and in this example the current is quite large. Typically, when observed, the maximum peak amplitude was between -100 and -500 pA. A full concentration response curve to TTX was performed on this cell revealing an IC_{50} for TTX of 5 nM (Figure 2). The voltage-gated Na^+ current is TTX sensitive although the exact Na^+ channel subtype present was not yet investigated further.

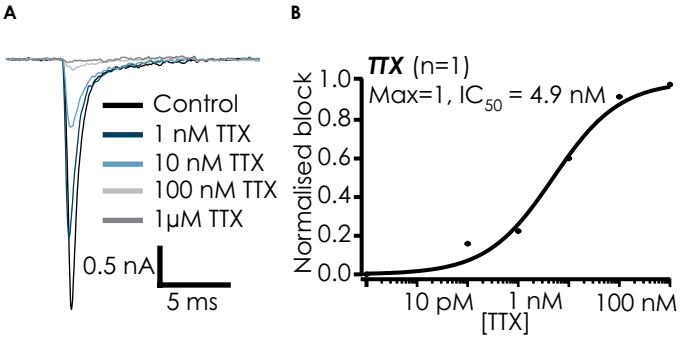

Figure 2:
A Block of Na^+ current by increasing concentrations of TTX. Current was blocked by low nM concentrations of TTX indicating a TTX sensitive Na^+ channel type expressed in this cell. **B** Concentration response curve for TTX inhibition, IC_{50} = 4.9 nM (n =1).

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An outward K^+ current was also seen in some cells. An example of the K^+ current recorded is shown in Figure 3. The current could be blocked by approximately 50% by 1 mM TEA (data not shown).

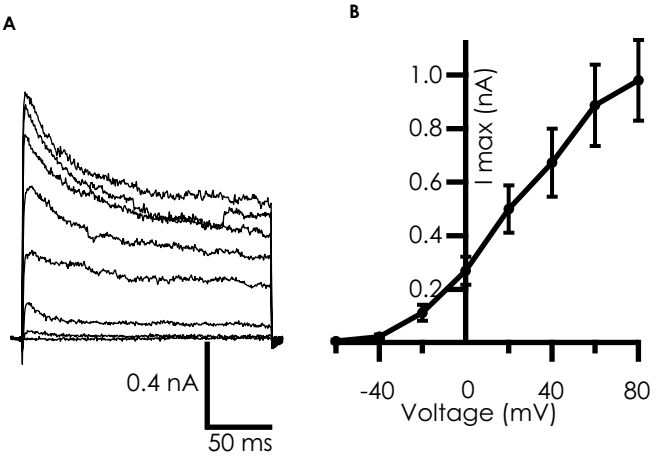

Figure 3:
A Voltage-gated K^+ current recorded in iCell® neurons. Current responses to a voltage step protocol. An outward K^+ current can be seen in this cell. **B** Corresponding IV plot from an average of 7 cells.

To investigate the expression of ligand-gated ion channels in iCell® Neurons, a single concentration of GABA (30 µM) was applied to the cells for ~500 ms (Figure 4). The current could be blocked by ~50% by 1 µM bicuculline.

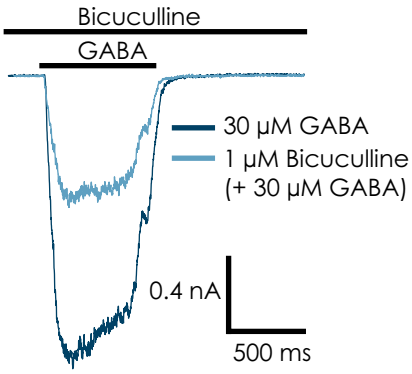

Figure 4:
Activation of GABA currents by 30 µM GABA and partial block of the GABA response by 1 µM bicuculline. Bicuculline was pre-applied for at least 30 s before co-application with GABA (30 µM). Approximately 50% of the current was blocked by 1 µM bicuculline.

In summary, iCell® Neurons from Cellular Dynamics International can be successfully used on the Patchliner® to combine a cellular neuronal model with higher throughput automated electrophysiology. Such a cell model provides an alternative to primary neuronal cell cultures for studying neuronal toxicity, disease research and drug discovery.

Methods

Cells

Human iPS cell-derived neurons (iCell® Neurons) from Cellular Dynamics International 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 a combination of trypsin and TrypLE.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner®. For Na^+ 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 TTX application, cells were stepped from -100 mV to -20 mV for 10 ms repeated every 5 s. For K^+ 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 GABA experiments, cells were held at a constant holding potential of -70 mV and GABA was applied for ~500 ms using a stacked solutions protocol. Bicuculline was pre-incubated for at least 30 s before co-application with 30 µM GABA.

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