Application Note

Ion Channels:
Na_V, K_V, $GABA_A$

Cells:
iCell® Neurons

Tools:
Patchliner

Stem cell derived neurons recorded on Nanion's Patchliner

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Summary

Stem cell–derived neurons provide a novel and unique model for studying human drug targets in their physiologically relevant environment of terminally differentiated, postmitotic cells. It has been increasingly recognized that associated proteins modulate the physiology and pharmacology of neuronal proteins[1,2]. Therefore, assays that investigate neuronal toxicity, drug effects, or basic cellular functions of neurons can mostly benefit from the development of human neurons derived from induced pluripotent stem cells (hiPSC-neurons). In 2011, Fujifilm Cellular Dynamics International (CDI) announced the commercial launch of human iCell® Neurons for use in neuroscience drug discovery, neurotoxicity screens, and other health research. This was the first commercially available iPSC-derived neuronal type available[3]. It was a mixture of GABAergic and glutamatergic post-mitotic neurons which rapidly regenerate post-thaw[4]. Over the years, Fujifilm Cellular Dynamics International has developed a range of hiPSC-neurons with either healthy or diseased models. These can be used to study a number of different neurodegenerative diseases including Alzheimer's Disease, one of the leading causes on death in the United States[5].

iCell® Neurons were the first commercially available hiPSC-neuronal cell type which could be provided in the quantity, quality, and purity required for life science research. These neurons were used on the Patchliner, a fully automated patch clamp device for recording from up to 8 cells simultaneously. 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 presence of Na_V, K_V and $GABA_A$ receptor mediated currents were detected in these cells and the results agreed well with manual patch clamp data using the same cells[6].

Results

Voltage-gated Na^+ (Na_V) currents of an individual cell are shown in Figure 1A. Not every cell showed expression of a Na_V current and in this example the current is quite large. Typically, when observed, the maximum peak amplitude was between -100 and -500 pA. The current voltage plot of 54 cells is shown in Figure 1B. A full concentration response curve to TTX was performed on 5 cells, revealing an IC_{50} for TTX of 12 ± 6 nM (Figure 1C,D). The Na_V current is TTX sensitive although the exact Na^+ channel subtype present was not yet investigated further.

An outward voltage-gated K^+ (K_V) current was also seen in most cells. There appeared to be two populations of K_V currents, a

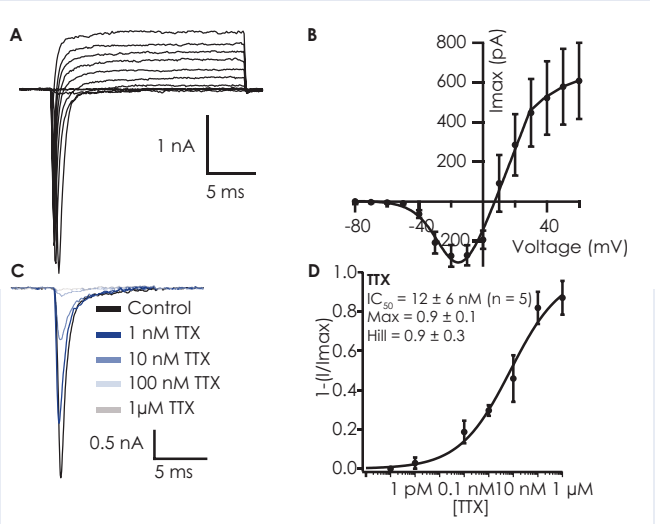

Figure 1: A *Na_V current recorded from an example iCell® Neuron.* **B** *IV plot from an average of 54 cells.* **C:** *Na_V current blocked by increasing concentrations of TTX.* **D** *Concentration response curve for TTX inhibition for an average of 5 cells.*

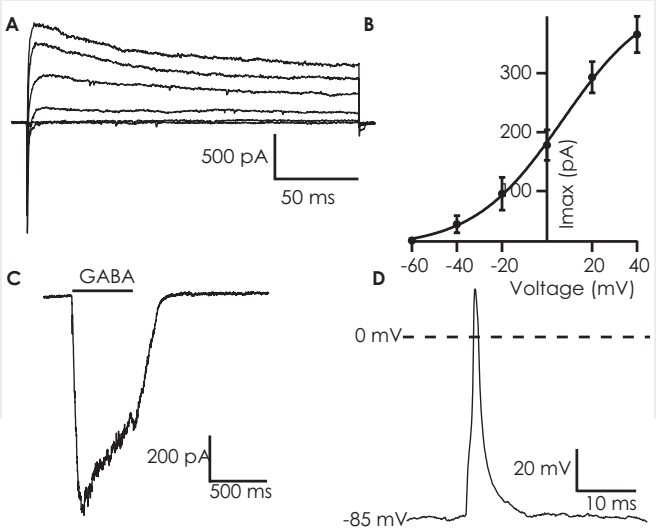

Figure 2: A *Inactivating K_V current recorded from an iCell® neuron.* **B** *Corresponding IV plot from an average of 121 cells.* **C** *$GABA_A$ receptor mediated current activated by 500 ms application of 30 µM GABA.* **D** *Action potential elicited from an iCell® neuron following a 2 ms depolarizing current pulse.*

non-inactivating (not shown) and an inactivating type (Figure 2A), although in most cases it was not possible to clearly distinguish the 2 populations[6]. A current-voltage plot for an average of 121 cells is shown in Figure 2B. The current could be blocked by increasing concentrations of TEA (data not shown)[6] but was not fully blocked even with the highest concentration of TEA (10 mM)[6]. $GABA_A$ receptors were activated in iCell® Neurons using a single concentration of GABA (30 µM) applied to the cells for ~500 ms (Figure 2C). The current could be blocked by increasing concentrations of bicuculline with an IC_{50} = 632 ± 149 nM (n = 5)[6]. In current clamp mode, action potentials were elicited using a 2 ms depolarizing current pulse (Figure 2D).

In summary, iCell® Neurons from Fujifilm Cellular Dynamics International can be successfully used on the Patchliner to combine a cellular neuronal model with higher throughput automated electrophysiology (see Ref. 6). Such a cell model provides an alternative to primary neuronal cell cultures for studying neuronal toxicity, disease research and drug discovery.

References

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Methods

Cells

Human iPS cell-derived neurons (iCell® Neurons) from Cellular Dynamics International were used.

Electrophysiology measurements

Cells were received as frozen aliquots and were plated and cultured according to the manufacturer's instructions. Cells were harvested according to Nanion's standard protocols. Cells were resuspended in external recording solution and stored in the CellHotel of the Patchliner before being dispensed into each well of the NPC-16 chip. Internal and external solution compositions are available upon request. 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 TTX application, cells were stepped from -100 mV to -20 mV for 10 ms repeated every 5 s. 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 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. For current clamp recordings, cells were switched from voltage clamp to current clamp mode using the 'gentle switch' method of the HEKA EPC10 amplifier. Action potentials were elicited using a 2 ms depolarizing current pulse.

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