

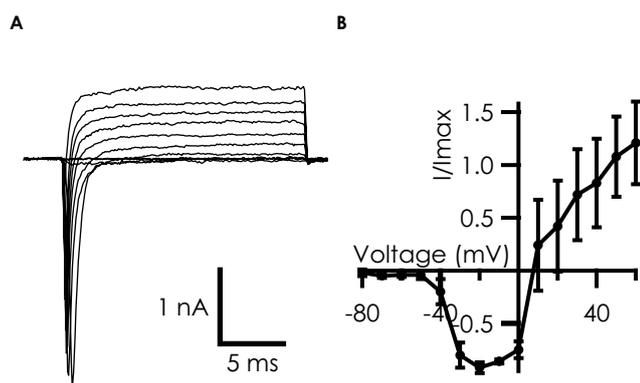
## Stem cell derived neurons recorded on Nanion's Patchliner®

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### 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.

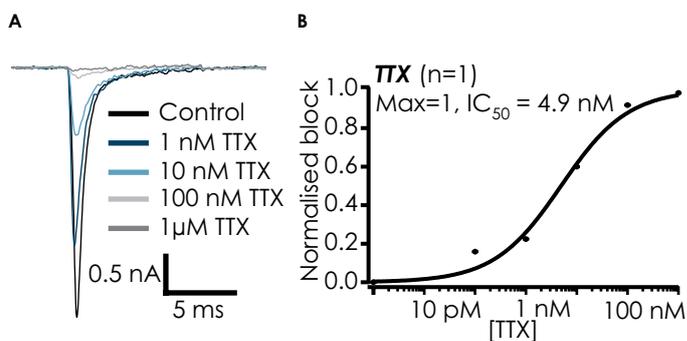


**Figure 1:**

**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.

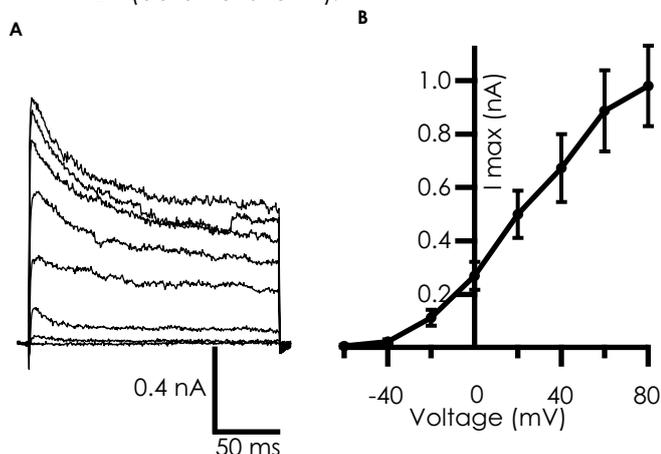


**Figure 2:**

**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 ( $n = 1$ ).

# Application Note

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<sup>®</sup> 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<sup>®</sup> Neurons, a single concentration of GABA ( $30 \mu\text{M}$ ) was applied to the cells for  $\sim 500$  ms (Figure 4). The current could be blocked by  $\sim 50\%$  by  $1 \mu\text{M}$  bicuculline.

## Methods

### Cells

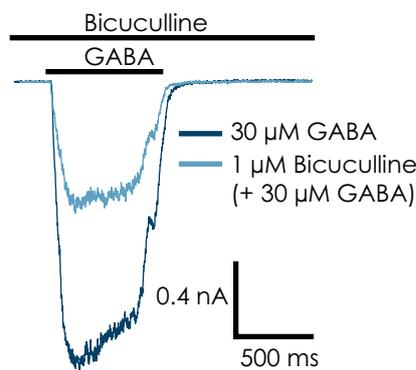
Human iPS cell-derived neurons (iCell<sup>®</sup> Neurons) from Cellular Dynamics International were used.

### 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<sup>®</sup>. For  $\text{Na}^+$  currents, cells were stepped from a holding potential of  $-100$  mV to  $-80$  mV for 20 ms and then



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

In summary, iCell<sup>®</sup> Neurons from Cellular Dynamics International can be successfully used on the Patchliner<sup>®</sup> 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.

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  $\sim 500$  ms using a stacked solutions protocol. Bicuculline was pre-incubated for at least 30 s before co-application with  $30 \mu\text{M}$  GABA.