Application Note

Ion Channels:
Na_V, $GABA_A$, glutamate, P2X

Cells:
ioSensory Neurons

Tools:
Patchliner

Characterization of ioSensory Neurons on the Patchliner

Nanion Technologies GmbH, Munich
bit.bio, Cambridge, UK

Summary

Human induced pluripotent stem cells (hiPSCs) are increasingly used in biomedical research for disease modeling 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 the requirement to culture the neurons for long time periods to achieve maturity. This can pose a potential challenge when combining hiPSC-derived neurons with techniques such as automated patch clamp (APC) which require cells in suspension for the experiments. When cells are cultured for longer (4 weeks or more) this can result in lower success rates for cell capture, and therefore, overall success rate for completed experiments. bit.bio uses opti-ox (optimised inducible overexpression) technology for cell programming. With this technology, genetic programs in human stem cells can be faithfully executed, leading to precise and consistent programming of the population into a chosen cell identity at a commercial scale. ioSensory Neurons (bit.bio, Cambridge, UK. Catalogue number io1024) are >99% pure sensory neurons with a defined nociceptor identity by day 7 post-revival, as confirmed by single cell RNA sequencing. For example, the expression of key nociceptor marker genes (NTRK1 and TRPV1) could be detected in post-mitotic sensory neurons. The ioSensory Neurons have been shown to be spontaneously active using the multi-electrode array (MEA) technique between 6 and 17 days in culture (DIV), and calcium imaging confirms the presence of TRPV1, TRPM3 and TRPM8[1].

In collaboration with bit.bio we cultured ioSensory Neurons for 14 - 21 days and characterized ion channel currents on the Patchliner.

Results

ioSensory Neurons were thawed and cultured as per the manufacturers instructions. The neurons were harvested at day 14 or 21 after thawing for use on the Patchliner. When added to the NPC-16 chip, ioSensory Neurons were captured in 100% of wells (100% capture rate) and following cell sealing, >80% of cells achieved a R_{Seal} of >500 MΩ and could be used for voltage and current clamp measurements (Figure 1, Table 1).

	Cm (pF)	Na_V peak (TTX-s;nA)	Na V_{half} (TTX-s; mV)	Na_V peak (TTX-r;nA)	Na V_{half} (TTX-r; mV)
14 DIV	4.9 ± 0.6 (13)	-2.5 ± 0.5 (13)	-25.8 ± 2.2 (13)	-0.2 ± 0.1 (9)	-4.3 ± 6.4 (9)
21 DIV	4.4 ± 0.6 (10)	-2.2 ± 0.6 (10)	-31.8 ± 3.0 (9)	-0.3 ± 0.2 (3)	-30.9 ± 2.3 (3)

Table 1: Cell capacitance and current amplitudes of Na_V currents at different time points.

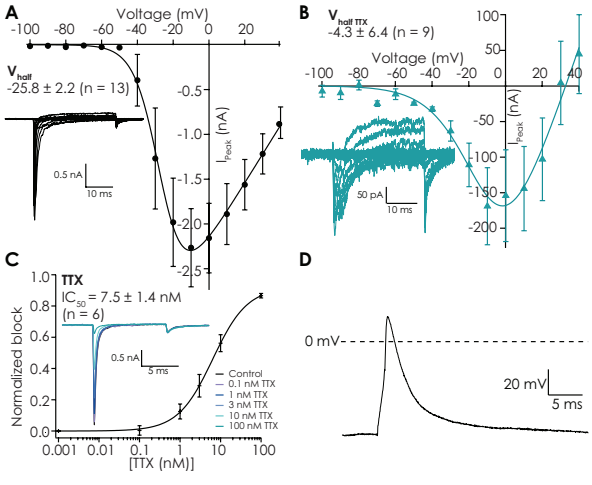

Figure 1: A *Average Na_VIV in control conditions for n = 13 ioSensory Neurons.* **B** *Average Na_VIV in the presence of 100 nM TTX. Currents are smaller and V_{half} of activation is shifted to more positive potentials.* **C** *Concentration response curve for TTX for an average of 6 ioSensory Neurons.* **D** *Example action potential elicited using a brief current pulse.*

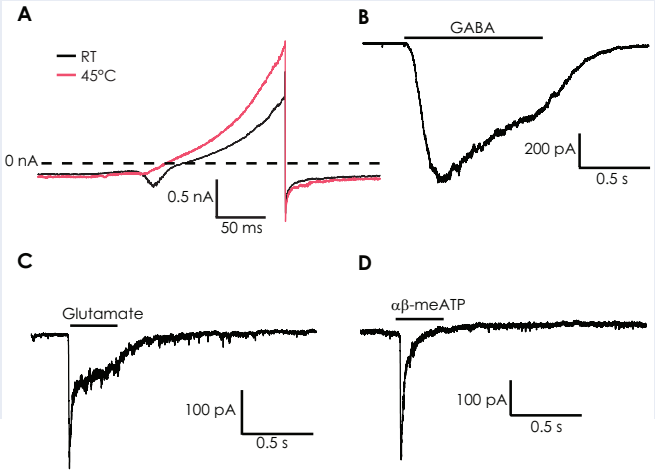

Figure 2: A *A heat-activated response was recorded in ioSensory Neurons when solution at 45°C was applied to the cell.* **B** *$GABA_A$ receptor-mediated responses were recorded in response to 100 µM GABA.* **C** *Ionotropic glutamate receptor responses were recorded after activation with 1 mM glutamate.* **D** *P2X receptors were recorded after addition of 30 µM* α,β-methylene ATP.

Peak current amplitude and V_{half} of activation of the TTX-sensitive (TTX-s) Na_V was similar at 14 and 21 DIV (Figure 1, Table 1, no statistical differences using Student's t test). The majority of the Na_V current was blocked by 100 nM TTX, with approximately 200 - 300 pA TTX-resistant Na_V current remaining. The TTX-r current had a V_{half} activation of -4.3 ± 6.4 mV (n = 9) but due to the large spread of data and low cell number, experiments should be repeated to make a comparison at the different time points. The IC_{50} for TTX of the TTX-s current was 7.5 ± 1.4 nM (n = 6). As no differences were observed between 14 and 21 DIV (P>0.05, Student's t test), data was pooled for the TTX concentration response curve (Figure 1C). When cells were switched to current clamp mode, action potentials could be elicited using a brief current pulse (Figure 1D).

In order to test for heat activated and ligand activated currents, we sequentially applied a number of ligands. A heat -activated response was observed in 12/13 cells when heated solution at 45°C was applied (Figure 2A). In a subset of neurons (approx. 50%) we were able to elicit a response to 100 µM GABA (Figure 2B), in 100% of neurons we could elicit a response to 1mM glutamate (Figure 2C) and in 20-50% we could elicit a P2X-mediated response to α,β-methylene ATP (α,β-meATP; Figure 2D). No responses were recorded in response to Yoda1 (10 µM).

In summary, ioSensory Neurons (bit.bio) could be used on the Patchliner with excellent success rates for cell capture and sealing. TTX-s and TTX-r Na_V currents were recorded and several ligand-gated ion channels could be detected. When current clamp mode was used, action potentials could be elicited.

References

1. ioSensory Neurons: Highly pure human iPSC-derived sensory neurons with a defined nociceptor identity (accessed 24.09.2024)

Methods

Cells

ioSensory Neurons (Catalogue number io1024. bit.bio Limited, Cambridge, UK.) were kindly provided by bit.bio and were thawed and cultured according to the User Guide.

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 CellHotel of the Patchliner 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 Patchliner. Activation IVs were recorded using a voltage step protocol and analyzed using Igor (wavemetrics). TTX was applied in increasing concentrations using a single step protocol to 0 mV and analyzed in Igor. For the heat activation the external solution in the pipette was heated to 45°C and rapidly applied to each well during a voltage ramp protocol from -100 mV to +100 mV. Ligands GABA, glutamate, α,β-meATP and Yoda1 were applied using the stacked solution approach and rapidly applied to the cells at a constant holding potential of -100 mV. Action potentials were elicited in current clamp mode with a 1 ms current pulse to 200 pA.