Application Note

High throughput APC recordings from RealDRG™ iPSC sensory neurons for analgesia drug discovery

Ion Channels:
Na_V, Ca_V, K_V
Cells:
RealDRG™ hiPSC-derived sensory neurons
Tools:
SyncroPatch 384
Patchliner

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Summary

There is great need for more effective, non-addictive analgesics due to poor efficacy and narrow therapeutic index of current treatments, and the desire to replace opioids due to the ongoing addiction and abuse crisis. Analgesia drug discovery still relies on animal models that poorly predict efficacy in human patients, leading to costly clinical failures for validated pain targets. Access to human dorsal root ganglion (DRG) reagents promises superior translation, but donor human tissue is limited and expensive. In contrast, human iPSC sensory neurons offer a scalable, flexible and cost-effective analgesia drug discovery reagent amenable to many screening platforms[1,2]. These human DRG reagents will also help meet 3Rs guidelines to reduce animal use and are compatible with the FDA Modernization Act 2.0 that will allow drug approval without animal testing.

Here we demonstrate that hiPSC sensory neurons are ideally suited for automated patch clamp (APC) recordings, as:

- Their large soma and simple dendritic architecture are retained after dissociation and cell suspension
- Capacitance measurements allow categorization of different sensory neuron phenotypes by cell size
- High throughput sampling from large numbers of cells enables statistical analysis of sensory cell types
- High fidelity recordings provide excellent voltage control in single patch-clamped cells
- Ionic currents can be reliably studied using complex voltage clamp protocols
- This technique allows current clamp recordings of single action potentials and membrane excitability

RealDRG™ human iPSC sensory neurons rapidly mature to express nociceptor markers such as TRPV1, P2X3, $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$[2]. APC voltage clamp recordings on the Patchliner and SyncroPatch 384 revealed voltage-gated K_V and TTX-sensitive (TTX_s) and TTX-resistant (TTX_r) inward Na_V channels, and overshooting single and repetitive action potentials under current clamp. This validation data confirms that Nanion APC platforms and Anatomic's hiPSC sensory neurons can be combined for reliable and scalable high throughput patch clamp studies of human pain signalling targets and peripheral neurotoxicity screening.

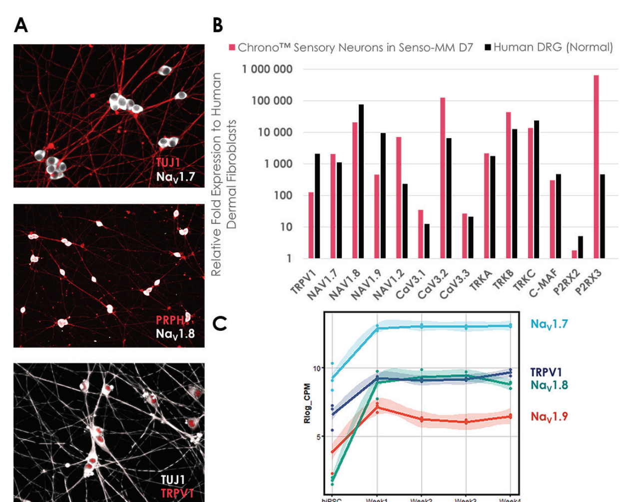

Figure 1: *Ion channel expression in RealDRG™.* **A** *Immunocytochemistry characterization of RealDRG™.* **B** *Comparison of expression of ion channels in RealDRG™ with human DRG.* **C** *RNA sequencing data reveals expression of genes SCN9A, SCN10A and SCN11A encoding $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$, respectively, along with TRPV1.*

Results

Human stem cell-derived sensory neurons offer many translational and ethical advantages over current preclinical animal tissue and *in vivo* models and human donor tissue, as they offer easy and affordable access to highly pure, consistent, transferable and scalable cell-based assays with commercial freedom-to-operate. RealDRG™ hiPSC sensory neurons can be rapidly differentiated and matured using a validated protocol and manufacturing process that works across multiple hiPS donor cell lines[1]. Neural crest is induced from primary ectoderm and then differentiated using small molecules and growth factors, before the sensory neurons are frozen and shipped to users for thawing and further maturation as desired. Extensive molecular validation shows that expected sensory neuron markers such as ISLET1, TUJ1, BRN3A, PRPH and various TRK neurotrophin receptors are prominently expressed by Day 7, alongside key analgesia ligand- and voltage-gated ion channels such as TRPV1, $P2X_3$, and various $Na_V1.x$ and $Ca_V3.x$ ion channels whose expression is maintained for 4 weeks *in vitro* and which achieve expression levels similar to that seen in adult human DRG (Fig. 1). Of the key 173 evolutionarily-conserved human DRG-specific gene markers, over 90% are expressed in RealDRG™ iPSC sensory neurons, whereas only 63% are present in mouse DRG, highlighting the species differences in this ganglia[3]. The remaining 10% of genes relate to myelination and immune cells, and their absence reflects the fact that these sensory neuron cultures are > 95% pure (allowing users to add native or iPSC astrocytes and microglia to their end-stage assays). RealDRG™ hiPSC neurons recapitulate the developmental patterns and maturation of DRG into varied types of sensory neurons[4], including characteristic gene expression patterns typical of human A and C-fibres (e.g. Aβ and Aδ mechanoreceptors, and PENK+, Aβ and silent nociceptors).

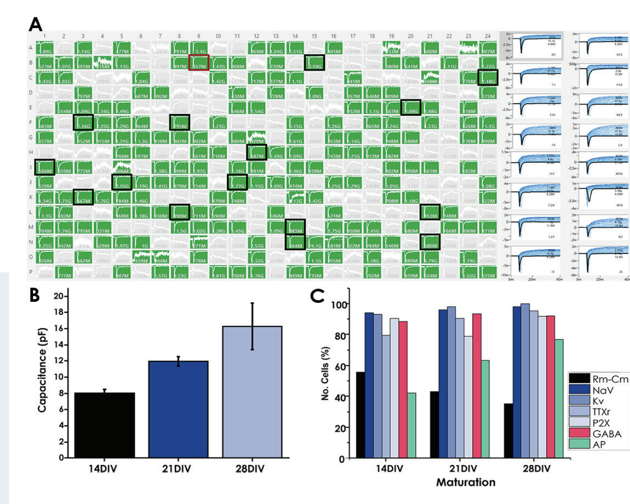

Figure 2: *Success rates on the SyncroPatch 384.* **A** *Screenshot of an experiment showing >50% of wells have a cell captured.* **B** *Cell capacitance increases with time in culture (DIV).* **C** *Success rates for cell capture, recordings of ion channel currents and eliciting action potentials (AP) at 14, 21 & 28 DIV.*

RealDRG™ hiPSC sensory neurons can produce high throughput, high quality recordings on APC platforms with excellent success rates, surpassing that seen with native rodent DRG neurons. Seal quality is excellent (>300 MΩ), and overall quality control pass rates range between 35-60% for cells matured over 14-28 days *in vitro* (DIV), meaning that high quality data from over 100 sensory neurons can be obtained in less than an hour from each NPC-384 chip on the SyncroPatch 384. Voltage-gated inward Na_V and outward K_V currents and functional P2X and $GABA_A$ ligand-responses are seen in 80-100% of cells, while action potential firing increases from 40 to 80% of cells over 3 weeks (Table 1).

DIV	QC	Na_V	K_V	TTXr	P2X	GABA	AP
14	56	94	93	80	91	88	42
21	43	96	98	91	79	94	63
28	35	98	100	96	92	92	77

Table 1: *Amount of successful wells (%) at different time points (14, 21 and 28 DIV).*

DRG sensory neurons express a wide range of voltage-gated inward Na_V and Ca_V, and outward K_V currents that control pain transmission, which can be accurately measured under voltage clamp using APC. TTX-sensitive $Na_V1.7$ and TTX-resistant $Na_V1.8$ and $Na_V1.9$ channels are of particular interest for analgesia, as mutations in these targets are associated with various pain states in human patients[5]. RealDRG™ sensory neurons express SCN9A and SCN10A RNA and protein, with lower levels of SCN11A, and IV curves applied on the SyncroPatch 384 and Patchliner show large TTX-sensitive currents activating at negative potentials, and a significant (~10 - 20%) component of TTX-resistant inward current that activates at more positive potentials, characteristic of $Na_V1.8$ channels (Fig. 3). The kinetics of TTX-resistant currents in RealDRG™ iPSC sensory neurons, and their relative contribution to macroscopic I_{Na}, are similar to those recorded from adult human DRG[6] but differ from that in rat DRG neurons, illustrating

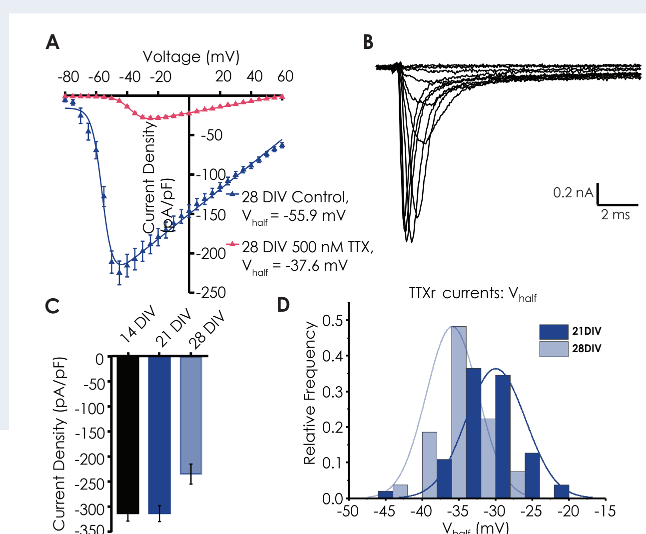

Figure 3: *Na_V currents in RealDRG™ sensory neurons.* **A** *IV plot at 28 DIV in the absence (blue) and presence of TTX (red) recorded on the SyncroPatch 384.* **B** *Raw current traces from an example cell in the presence of TTX.* **C** *Na_V current density decreases during maturation (total Na_V current), and the V_{half} of activation of the TTX_r current becomes more negative (**D**).*

the species differences in many sensory neuron responses and importance of using human cells for target validation and translation.

RealDRG™ sensory neurons also express TTX-sensitive Na_V1.2 and Na_V1.6 channel genes which are found in mature rodent and human DRG neurons, as well as low levels of the embryonic Na_V1.3 channel which is up-regulated after nerve injury[5]. Na_V1.5 is the third TTX-resistant channel normally expressed in neonatal and adult DRG neurons (especially in human Aδ mechanoreceptors), and is downregulated during *in vitro* maturation of RealDRG™ iPSC sensory neurons as occurs *in vivo*.

DIV	Spontaneous APs (% cells)	Elicited APs (% cells)
14	0.4	42
21	3.3	63
28	3.5	77

Table 2: *Spontaneous action potentials were observed in <5% of cells but could be elicited in >40% of cells, increasing to 77% with culture time.*

It is also possible to use APC to make high throughput current clamp recordings from hiPSC sensory neurons, even in the same cells used for voltage clamp experiments to correlate ionic current expression and pharmacology with neuronal excitability. Baseline membrane potential recordings show a moderate resting membrane potential and a low level of spontaneous action potential (AP) firing, as expected for DRG neurons (Table 2). Spontaneous firing is very low after 14 DIV, but increases to occur in 3% of sensory neurons after further maturation. Single and repetitive APs can be elicited with depolarizing current injections on the SyncroPatch 384 and Patchliner (Fig. 4), enabling categorization of single sensory neurons by their AP shape, pharmacology and evoked AP trains. Application of current steps or ramps can also be used to assess changes in sensory neuron excitability, a key driver of pain signalling.

Discussion

These data demonstrate the utility of RealDRG™ human iPSC sensory neurons to bridge the translational gap in analgesia R&D, and their compatibility with APC platforms. High quality recordings of Na_V currents can be obtained with high success, with Na_V channel biophysics and pharmacology indicating the presence of large TTX_s currents (likely Na_V1.7), alongside a significant component of TTX_r current which transcriptome and pharmacology data suggest is carried by Na_V1.8 channels. Na_V1.7 and Na_V1.8 are important analgesia drug discovery targets based on human pain channelopathies and remain the focus of industry efforts to develop sub-type selective small molecules, mRNA and biologic ligands to treat pain. It is also possible to record firing of single and repetitive action potentials on APC platforms, and assess changes in sensory neuron membrane excitability with complex current clamp protocols which can be useful for phenotypic analgesia drug screening. The low rate of spontaneous AP firing, together with the lack of an extensive dendritic network and synaptic connections, makes it more difficult to achieve high throughput recordings from sensory neurons on multi-electrode arrays compared to CNS neurons, making APC a promising platform for analgesia drug testing and neurotoxicity screening with RealDRG™ hiPSC sensory neurons. The ability to achieve good voltage control and quickly obtain complex datasets from hundreds of single cells will facilitate mechanistic understanding of drug actions, and the plasticity of RealDRG™ hiPSC sensory neurons means that novel maturation protocols can be applied to direct the development of specific DRG sensory neuron subtypes relevant to analgesia research.

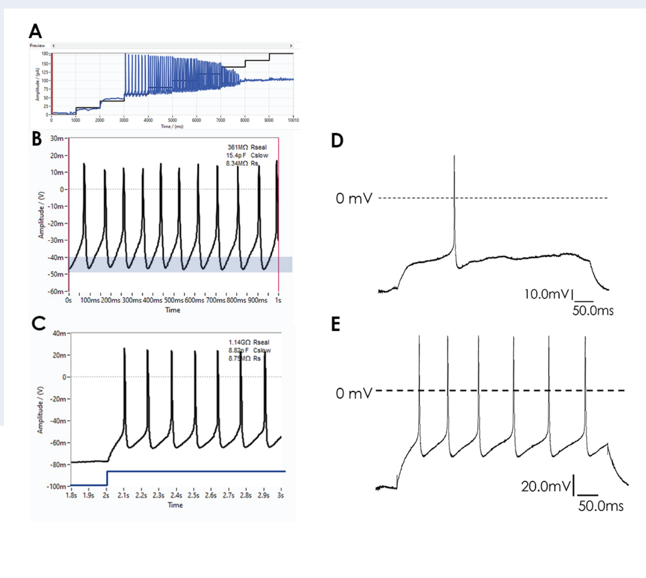

Figure 4: *Action potentials in RealDRG™ sensory neurons.* **A** *Current step protocol to elicit action potentials.* **B** *Spontaneous APs recorded on the SyncroPatch 384.* **C** *Elicited APs using a current step to 40 pA.* **D & E** *Elicited APs on the Patchliner. In some cells a single AP was elicited* **(D)** *whilst in others a train of APs was elicited during the 500 ms current pulse.*

References

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2. Li *et al.* (2021) Front Cell Dev Biol 14 (9): 796960
3. Ray *et al.* (2018) Pain 159: 1325–1345
4. Tavares-Ferrara *et al.* (2022) Sci. Transl. Med. 14: eabj8186
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6. Zhang *et al.* (2017) e-Life 6: e23235

Methods

Cells

RealDRG™ iPSC sensory neurons were differentiated according to manufacturers protocols and frozen after 7 days.

Electrophysiology

Cells were thawed and plated into T75 flasks coated with iMatrix 511-SILK at a seeding density of 40,000 cells per cm^2 and matured in Senso-MM before being gently dissociated and resuspended in external recording solution and stored in the CellHotel on the Patchliner or SyncroPatch 384 APC platform, before being dispensed into each well of the NPC-16 or NPC-384 chip, respectively. Standard patch clamp electrophysiology internal (KF-based) and external solutions were used to record inward Na_V and outward K_V currents and action potentials, and Na_V currents were isolated by using a CsF-based internal solution. Standard voltage and current clamp protocols were used to set holding potential and measure resting membrane potential, and voltage-gated currents and action potentials were evoked with step voltage or current pulses.

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