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Patch clamp profiling the functional properties of human iPSC-derived nociceptors

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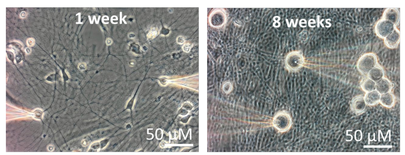
Abstract-

- According to the National Institute of Neurological Disorders and Stroke, 100 million adults in the United States suffer from chronic pain. One way to study pain is to use human iPSC-derived nociceptors as an in vitro model, but it is important to characterize the cell properties to understand the iPSC model.
- We recorded from over 100 Anatomic RealDRG™ hiPSC-derived nociceptors from 1-8 weeks in vitro.
- Human nociceptors plated at low density on rat astrocytes appeared healthier and were easier to patch compared to neurons plated without astrocytes. Nociceptors grew larger with time in culture as evidenced by an increase in Cm, with decreased Rm. The RMP became more hyperpolarized after three weeks and remained stable thereafter.
- We observed little spontaneous activity in cells at every developmental timepoint, as would be expected from sensory neurons. The nociceptors were excitable, however, as action potentials were elicited with depolarizing current injections. Further, the amount of current injection required to elicit the first action potential (rheobase) increased over developmental time, as expected from neurons with lower Rm.
- Additionally, nociceptors at all developmental timepoints exhibited sodium currents. The voltage-dependent sodium current amplitudes increased with time in culture, while the peak sodium current density (pA/pF) remained stable. We also evaluated the presence of TTX-R sodium currents at 5-8 weeks in vitro and detected their presence at all evaluated time points. The proportion of TTX-R among nociceptors varied.
- We also observed an increased spontaneous neuronal activity in sensory neurons with longer time *in vitro*, using HD-MEA.
- Overall, we determined that Anatomic RealDRGs® develop a mature neuronal phenotype by three weeks in culture, with hyperpolarized resting membrane potential, the ability to evoke spike trains, and exhibit TTX-sensitive and resistant Na currents.

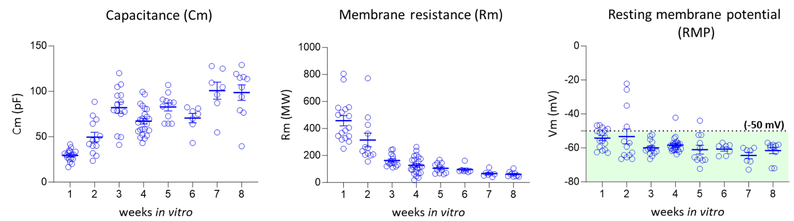
Methods-

- Anatomic RealDRGs® were thawed and plated at a low density, with a subset plated on a monolayer of rat astrocytes.
- Whole cell patch clamp recordings were performed on human iPSC-derived nociceptors at different timepoints (1-8 weeks) to determine the time course for the development of a mature neuronal phenotype.



- The composition of external recording solution was: 140 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1.3 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES pH 7.3. The composition of internal recording solution was: 120 mM K-gluconate, 20 mM KCl, 3 mM $MgCl_2$, 5 mM EGTA, 0.5 mM $CaCl_2$, 4 mM Na_2-ATP, 0.3 mM Li-GTP, 10 mM HEPES pH 7.3.
- Upon establishing the whole cell configuration, we measured the passive membrane properties for every cell. We also recorded spontaneous action potential firing, intrinsic excitability as well as evaluated the expression of voltage-gated sodium currents and tetrodotoxin (TTX)- resistant sodium channels (in presence of 0.5 µM TTX).
- We measured spontaneous action potentials using Maxwell MaxTwo high-density multielectrode array (HD-MEA) instrument.

1. Development of passive membrane properties of human-iPSC derived sensory neurons



Weeks in vitro	1	2	3	4	5	6	7	8
Cm (pF)	29.4 ± 1.6 (17)	49.6 ± 5.3 (13)	82 ± 6 (14)	67.1 ± 3.1 (24)	82.8 ± 4.4 (11)	70.5 ± 5.2 (7)	101 ± 9.4 (7)	98.6 ± 8.5 (10)
Rm (MW)	458 ± 38 (17)	315 ± 50.5 (12)	163 ± 12 (14)	127 ± 13 (24)	106 ± 11.4 (11)	96.4 ± 11 (7)	66.3 ± 8.6 (7)	61.4 ± 6 (10)
Vm (mV)	-54.2 ± 1.4 (17)	-53.3 ± 4.4 (13)	-60 ± 1.1 (14)	-58.3 ± 0.8 (24)	-61 ± 2.6 (24)	-61 ± 1.2 (7)	-64.5 ± 2 (7)	-62 ± 1.8 (10)

Figure 1: iPSC sensory neurons mature with longer time *in vitro*. Capacitance (Cm) increased and membrane resistance (Rm) with maturation time in vitro. A hyperpolarized resting membrane potential was established early. Values presented as average ± SEM (no. of neurons).

2. Majority of human iPSC sensory neurons showed low spontaneous activity

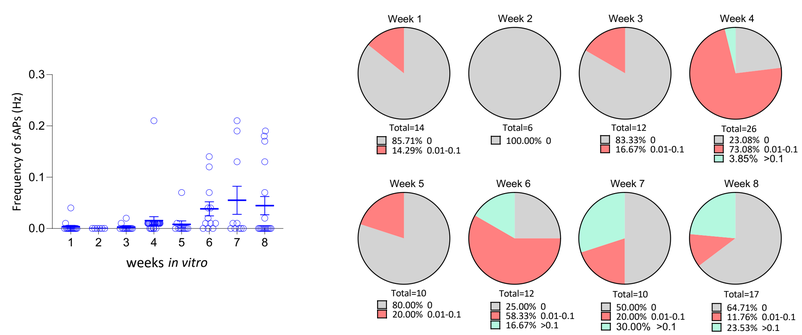


Figure 2: Majority of neurons do not fire spontaneous action potentials, as expected from sensory neurons. Current clamp recordings were performed at the neuron's resting membrane potential to assess spontaneous activity. Pie-charts represent percent of neurons exhibiting spontaneous action potential frequency at every timepoint.

Conclusions-

1. Human iPSC sensory neurons exhibited a mature developmental phenotype by 3 weeks *in vitro*, hyperpolarized resting membrane potential and ability to evoke action potentials.
2. Human iPSC sensory neurons elicited an increase in spontaneous neuronal firing as they mature in culture.
3. Human iPSC sensory neurons showed both TTX-S and TTX-R Na currents. Na current amplitude after TTX was large enough (~1000 pA) to test Nav 1.8 blocking compounds.

3. Development of intrinsic excitability of human-iPSC derived sensory neurons

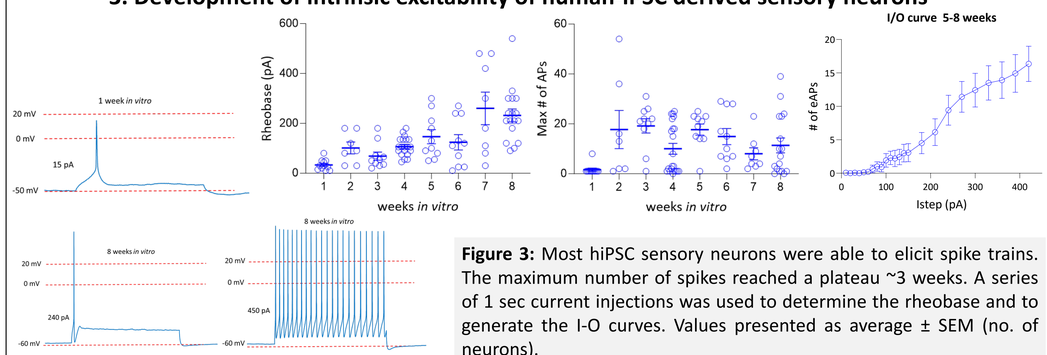
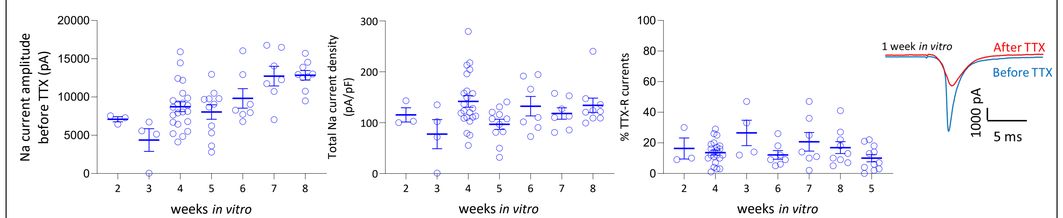


Figure 3: Most hiPSC sensory neurons were able to elicit spike trains. The maximum number of spikes reached a plateau ~3 weeks. A series of 1 sec current injections was used to determine the rheobase and to generate the I-O curves. Values presented as average ± SEM (no. of neurons).

Weeks in vitro	1	2	3	4	5	6	7	8
Rheobase (pA)	33.2 ± 6 (11)	101 ± 24 (7)	68 ± 16 (10)	107 ± 8 (20)	146 ± 28 (10)	124 ± 30 (10)	260 ± 65 (8)	232 ± 26 (17)
Max # of APs	2 ± 1 (11)	18 ± 8 (7)	19 ± 3 (10)	10 ± 2 (21)	18 ± 2 (10)	15 ± 3 (10)	8 ± 2 (8)	11 ± 3 (17)

4. Human iPSC sensory neurons exhibited both TTX-sensitive and TTX-resistant sodium currents



Weeks in vitro	2	3	4	5	6	7	8
Na current amplitude before TTX (pA)	7089 ± 575 (3)	4370 ± 1481 (4)	8730 ± 642 (22)	8034 ± 946 (11)	9823 ± 1276 (7)	12725 ± 1289 (7)	12834 ± 623 (9)
Total current density (pA/pF)	115 ± 14 (3)	78 ± 29 (4)	142 ± 11 (22)	97 ± 10 (11)	132 ± 19 (7)	118 ± 11 (7)	134 ± 14 (9)
% TTX-R	16 ± 7 (3)	26 ± 8 (4)	14 ± 2 (22)	10 ± 2 (11)	12 ± 3 (7)	21 ± 6 (7)	17 ± 4 (9)

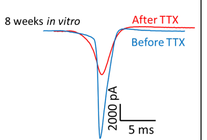


Figure 4: hiPSC sensory neurons exhibited increased sodium (Na) current amplitude over developmental time, which can be blocked by TTX application, yielding approximately 20% TTX-R currents. Values presented as average ± SEM (no. of neurons). Na currents were recorded before and after the application of 0.5 µM tetrodotoxin (TTX). Neurons were held at -80 mV potential and Vstep from -80 mV to +60 mV with 10 mV increment was performed every 100 ms.

5. Human iPSC sensory neurons showed increased spontaneous neuronal activity

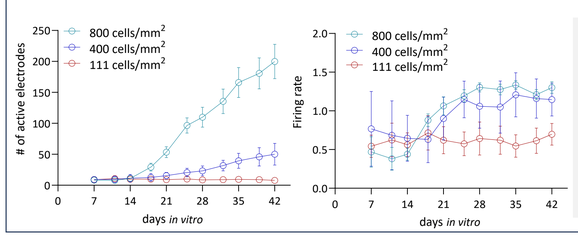


Figure 5: hiPSC sensory neurons exhibited increased spontaneous action potential firing over developmental time, measured using HD-MEA. The neurons were plated at high density without astrocytes. The number of active electrodes (neurons) was correlated to the plating density. The neurons plated at a higher density exhibited greater number of active electrodes (spontaneous activity).