APPLICATION NOTE

Functional characterization of human induced pluripotent stem cell-derived sensory neurons using a high-sensitivity micro-electrode array

Stem cell derived neurons offer valuable insights into drug discovery, drug safety testing, regenerative medicine, and the mechanisms of the brain, behavior, and disease. Neurons derived from human stem cells may provide physiological relevant applications for pharmacological assays that reflect human biology better than animals models. Neurons differentiated from stem cells demonstrate subtle yet diverse activity that can be intricate or robust. Due to the diversity of action potentials a cultured network of stem cell-derived neurons is capable of producing, especially in the presence of compounds and drugs, a high-sensitivity and diverse platform is needed to functionally characterize the activity. The MED64 Presto is a high-sensitivity high-throughput micro-electrode array (MEA) capable of fully characterizing electrophysiological activity in cultured stem cell derived neurons due to a broad acquisition bandwidth, industry leading signal-to-noise ratio, and electrodes engineered with carbon nanotube technology.

Materials and Methods

Sensory neurons that transduce pain are crucial to protect an organism from potentially damaging external stimuli. Dorsal root ganglions (DRG) are pain-related neurons that have a variety of sensory receptors that are activated by chemical, thermal, and mechanical stimulation. DRG neurons are invaluable tools for investigating the mechanisms of human pain sensation and developing drugs to treat or mitigate pain and other sensory disorders.

Extracellular electrophysiology was measured in human iPSCDerived Sensory Neuron Progenitors (ax0055, Axol Bioscience, UK) cultured at 5.0 × 10^5 cells/cm^2 on 384-channel 24 well MEA plates (Alpha Med Scientific) coated with Axol Sure Bond Coating Solution (ax0053, Axol Bioscience) at 37°C in a 5%CO_2, 95%O_2 air atmosphere. Immunofluorescent images were obtained by confocal microscopy (Leica TCS SP8).

MEA recordings were performed after 33 days in vitro (DIV) using the MED64 Presto 384 channel MEA (Alpha MED Scientific). Spontaneous activity and chemically evoked responses were measured at 37°C in an atmosphere controlled chamber (5% CO_2) at sampling rate of 20kHz at each electrode. Signals from each electrode were filtered using a 100 Hz high pass filter and raw data was logged using the MED Symphony software.

Optimization of MEA recordings

The MED64 Presto high-throughput MEA is engineered for sensitivity. With an industry leading signal-to-noise ratio (0.9 µV RMS) the MED64 Presto is capable of capturing a wide range of activity due to the high signal-to-noise ratio allowing for a broad acquisition bandwidth. The raw data signal does not need to be filtered to improve the signal-to-noise ratio so raw data logging is possible. The electrodes of the MED64 Presto multi-well MEA plates are engineered using carbon nanotube technology, a substrate that promotes neuron adhesion. Therefore, neurons will grow close to electrodes (see Figure 1), resulting in reliable and reproducible extracellular field potential recordings.

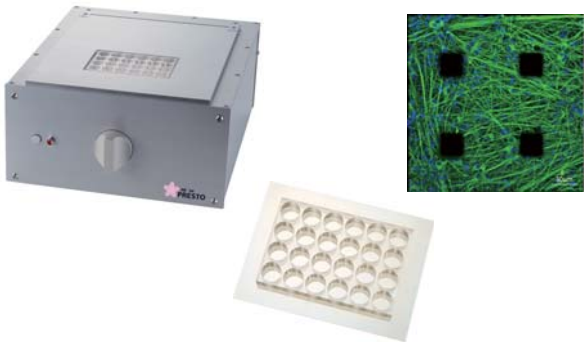

FIGURE 1: The MED64 Presto high throughput MEA (left) and Axol stem cell-derived sensory neurons plated onto MEA electrodes (right).

Evaluation of Functional activities

A functional evaluation of stem cell derive neurons should demonstrate that there is a physiologically relevant response that is specific to the subtype of neurons that are being evaluated. The unique biomarkers for stem cell-derived

Validation Data

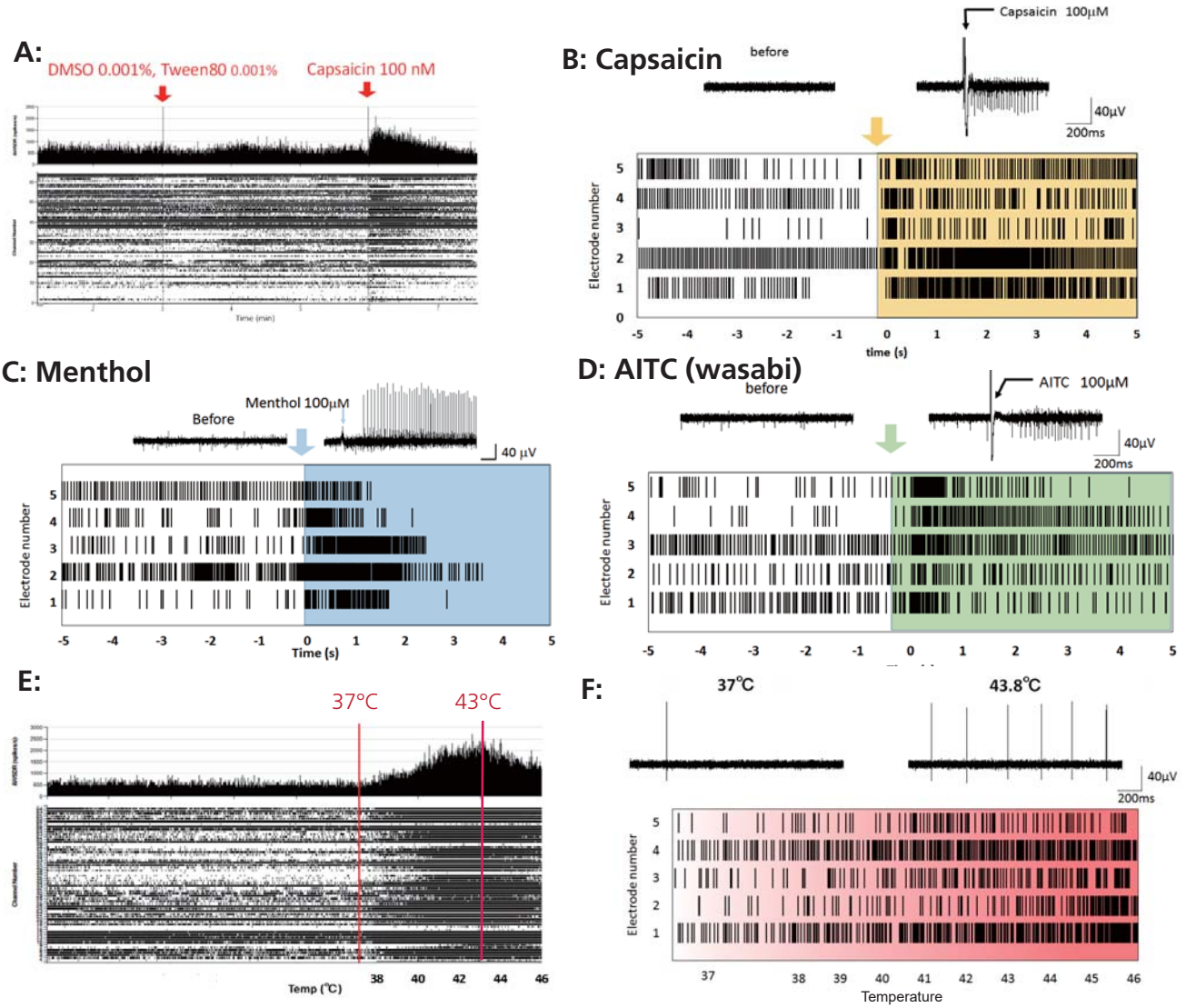

FIGURE 2: (A) Histogram (top) and raster plot (bottom) in response to vehicle control (DMSO) and after applying 100 nM Capsaicin. (B) Typical waveform and rasterplot in response to the application of 100 nM Capsaicin (C) Typical waveform and rasterplot in response to the application of 100 µM Menthol. (D) Typical waveform and rasterplot in response to the application of 100 µM AITC (wasabi). (E) Histogram (top) and raster plot (bottom) in response to change in temperature from 37 to 46 ℃. (F) Typical waveform and rasterplot in response to change in temperature from 37 to 46 ℃.

sensory neurons are the presence of the receptors TRPM8, TRPV1, and TRPA1. The presence of each receptor can be verified using immunohistochemistry by staining cells with proteins that bind to each receptor. Functional verification of each channel can be established pharmacologically by applying compounds that bind to each receptor and measuring a physiological response after applying the compound to the neuron culture.

The presence of NAV1.7, TRPV1, an TRPA1 were verified via immunostaining at 8 weeks in vitro (see Figure 3). The TRPV1channel was functionally verified by applying the TRPV1 agonist Capsaicin (100 nM) as well as change in temperature. The TRPM8 channel was functionally verified by applying Menthol (100 µM) and the TRPA1 channel was verified by applying AITC (wasabi, 100 µM). While the presence of each channel can be verified using immonustaining, electrophysiological response to pharmacological application of agonists can verify that each channel is physiologically active.

Spontaneous activities

Neurons derived from human iPSC-Derived Sensory Progenitors are spontaneously active (see figure 2). However, both the frequency of action potentials as well as the amplitude are small and sparse. Therefore, a high-sensitivity MEA is needed in order to reliably and reproducibly detect the sparse signal. The MED64 Presto is the ideal platform for functional assays because small sparse signals can be detected without the use of analogue or digital filters. With a poor signal-to-noise ratio, the sparse activity would be buried beneath the baseline noise level.

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Collectively, the presence of spontaneous activity as well as the ability to measure a subtle intricate signal make the MED64 Presto and Axol iPSC-Derived Sensory Progenitors the ideal toolkit for functional pain assays on sensory neurons.

Pharmacological induced activitis

When functionally evaluating the presence of specific receptors unique to a neuronal subtype, agonists are a useful probe as the agonist will increase activity of the neuron when applied to the neuron culture. The presence of channels specific to sensory neurons were verified by immunostaining (Figure 3) and the channels were verified as functionally active by observing an increase from baseline activity in response to agonist compound application (Figure 2).

Collectively, the physical presence of receptors specific to sensory neurons as demonstrated by immunostaining as well as the confirmation that those receptors are functional as demonstrated by increased activity in response to agonists indicates that Axol sensory neurons display typical characteristics of human sensory neurons.

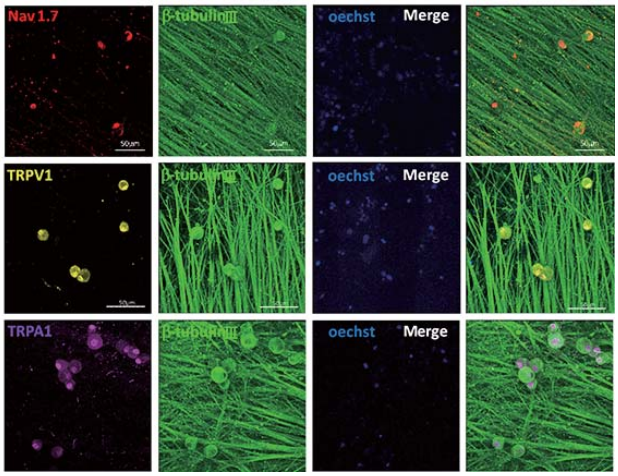

FIGURE 3: Immunostaining in cultured hiPSC-derived sensory neurons. Nav 1.7, TRPV1, TRPA1 expression at 8 weeks in vitro (WIV).

Functional Characterization

The MED64 Presto high-sensitivity high-throughput MEA coupled with Axol sensory neurons have been confirmed as the ideal toolkit for assessing pain-related responses in vitro. This study has verified that receptors indicative to sensory neurons are present and functional. Baseline spontaneous firing was observed in all experimental conditions. An increase in spike rate as well as an increase in amplitude of action potentials was observed in response to the application of Capsaicin and an increase in temperature. These results confirm that TRPV1 channels are present and functional (Figure 2A, 2B, 2E, and 2F). An increase in spike rate and amplitude of action potentials was observed in response to Menthol, confirming the presence and functionality of TRPM8 receptors (Figure 2C). An increase in spike rate and amplitude was observed in response to AITC (wasabi), confirming the presence and functionality of TRPA1 channels.

These results indicate that there are multiple ways to functionally characterize stem cell-derived neurons using the MED64 Presto. An increase in spike rate and the amplitude of spikes were observed. It is unlikely that the amplitude of spikes recorded extracellularly change significantly over the duration of an MEA recording. Therefore, an increase in spike rate for small amplitude spikes indicates that neurons that are spontaneously active increase their spike rate in response to agonists. An increase in the amplitude of the spikes suggests that neurons that were not spontaneously active under control conditions begin firing in the presence of agonists. Two different responses were observed, an increase in spike rate of spontaneously active cells and the initiation of spikes in nonspontaneously firing neurons.

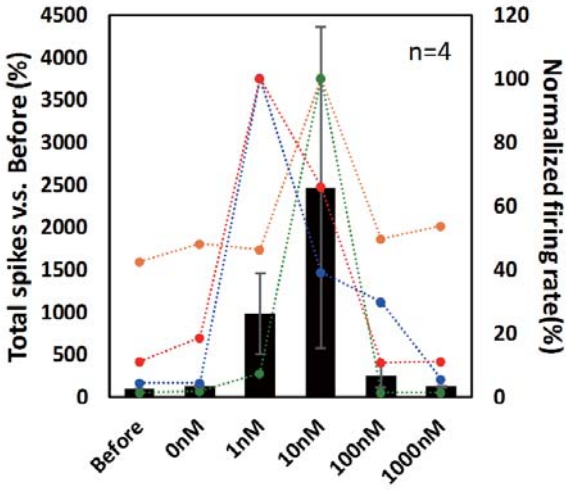

FIGURE 4: Firing change in oxaliplatin administration. N = 4 wells.

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Conclusion

The MED64 Presto is the most sensitive high-throughput MEA on the market. Sensitivity is engineered into the MED64 Presto system. High sensitivity provides superior signal-to-noise ratios which afford a broad acquisition bandwidth. A broad acquisition bandwidth enables the ability to record a diversity of responses from cultured cells. The ability to record the broad spectrum of possible action potentials from a neuron culture results in more reliable and more reproducible data.

This study demonstrated physiological responses to typical pain-related molecules (capsaicin, menthol, and AITC), temperature change in cultured hiPSC-derived sensory neurons and confirmed the expression of typical sensory neural markers. Our study shows that electrophysiological measurement in cultured hiPSC-derived sensory neurons using high-throughput MEA system are suitable to toxicological and drug screening assays in peripheral nerves. Figure 4 shows a response to the anti-cancer drug Oxaliplatin indicating that the MED64 Presto is a useful tool for evaluating pain response in drug discovery.

This study also demonstrated the power of coupling Axol sensory neurons with the MED64 Presto high-sensitivity high-throughput MEA in functionally characterizing pain response. In Figure 2, we demonstrated that multiple neurons were measured within the same assay. The fact that small amplitude as well as large amplitude spike were detected indicates that the MED64 Presto's broad acquisition bandwidth results in more reliable and more reproducible data from a diversity of neurons. Reliability and reproducibility is enhanced by the MED64 Presto's carbon nanotube technology resulting in better data for drug discovery, drug safety screening, regenerative medicine, and investigating the mechanisms of the brain, behavior, and disease.

All Data: provided by Ikruo Suzuki, PhD, Tohoku Institute of Technlogy

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