Novel neurotoxicity screening platform to examine functional changes in rodent dorsal root ganglia and human iPSC-derived sensory neuron cultures

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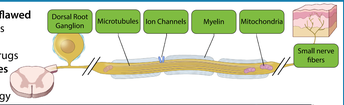
NerveSim® Powered by Nerve-On-A-Chip®

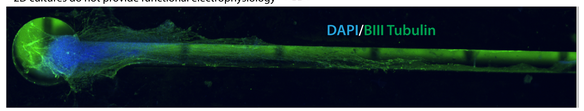
Overview

- **AxoSim has developed a novel microphysiological system (MPS), the NerveSim®, to model peripheral nerves *in vitro* using rat- or human-derived cells**
- **The platform uses an embedded electrode array (EEA) to record functional electrophysiological signals from peripheral nerve cultures**
- **NerveSim® is a scalable, automated platform allowing measurement of multiple clinically relevant electrophysiological metrics from compound action potential (CAP) recordings such as nerve conduction velocity (NCV), peak response amplitude (AMP), activity dependent slowing (ADS), and threshold stimulus strength (TSS)**
- **Quantification of these metrics enables compound screening for peripheral neurotoxicity, neuroprotection, and neurorehabilitation**

Introduction

- **The current neurological drug development pipeline is flawed**
  - 94% of neurological drugs fail at or before clinical trials
  - Drug development requires ~$2.6 billion per drug
  - Animal models are not translatable for neurological drugs
- **Peripheral neuropathy is hard to assess using 2D cultures**
  - Cannot simulate the long 3D growth *in vivo*
  - 2D cultures do not provide functional electrophysiology

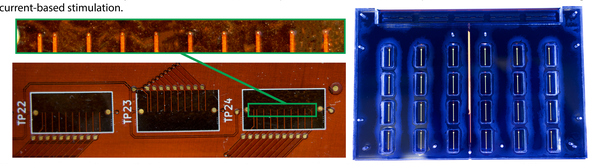




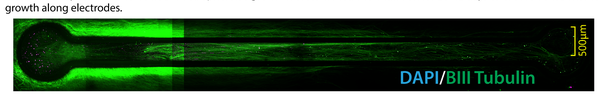
- **AxoSim has developed the NerveSim® platform, a novel peripheral nerve MPS using an EEA, as a scalable alternative for the pharmaceutical industry to measure functional electrophysiology from either rat or human models**
  - 3D tissue culture model to best mimic *in vivo* nerve growth
  - Designed to be scalable and automatable from the ground up
  - Collects clinically relevant electrophysiological metrics from primary rat or human induced pleuripotent stem cells (iPSCs) to maximize translation

Methods

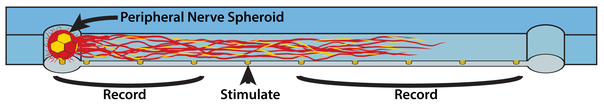
Embedded Electrode Arrays: Custom 24-well tissue culture plate with 10 microelectrodes per well that are used for recording or current-based stimulation.



Microphysiological System: Peripheral nerve spheroids are created from either primary rat dorsal root ganglia (DRG) cells or human induced pleuripotent stem cells (iPSCs). Polymer construct guides axonal growth of peripheral nerve spheroids along electrode array to mimic nerve fiber tract. Spheroids grown in custom media formulation for 28-42 days to ensure dense axonal growth along electrodes.

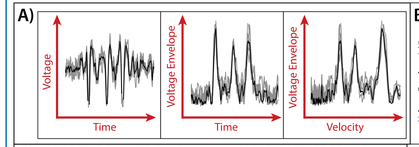


Compound Dosing: Positive control neurotoxic compounds are applied to mature NerveSim® cultures for 7-14 days, measuring electrophysiology before, during, and after dosing.



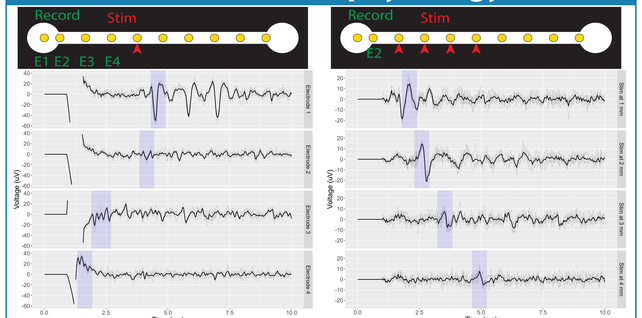
Electrophysiology: NerveSim® samples were stimulated with current-based electrical stimulation in parallel at multiple distal electrodes while recording on the remaining electrodes. Each stimulation used a stimulation current ramp (1 to 64 µA) to generate CAPs on nearby electrodes.

Data Analysis



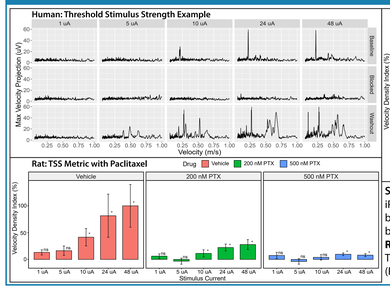
CAP Analysis: Raw signals were processed (A) and significant peaks were detected (B) for each electrode recording. Recordings from multiple electrodes in a single well were combined (C) into a maximal velocity projection (MVP) to quantify the population-level response to electrical stimulation for each individual NerveSim®. The area under the curve of the MVP was calculated to create a velocity density index (VDI).

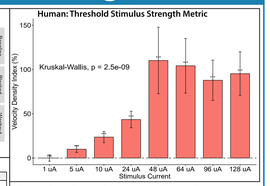
Evoked Electrophysiology



Example Responses: Raw electrophysiological recordings (**Left**) from a rat DRG NerveSim® stimulated at electrode 5 with 48 uA showing evoked responses on electrodes 1 through 4. Similarly, raw recordings (**Right**) from a human iPSC NerveSim® stimulated at electrodes 3 through 6 with 48 uA. The evoked responses on electrode 2 show latency shifts corresponding to stimulation distance with consistent velocities.

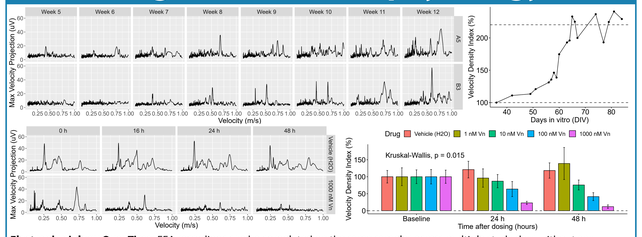
Threshold Stimulus Strength





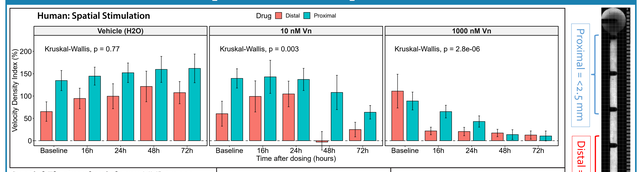
Stimulation Current Ramping: Stimulation of human iPSC NerveSim® with a current ramp (**Top Left**) before, during, and after application of a reversible blocker. A population current ramp response (**Top Right**) for human iPSC NerveSim® from 1 to 128 uA. TSS from a rat culture (**Left**) dosed with paclitaxel (PTX) showing differences across dosing conditions.

Longitudinal Electrophysiology

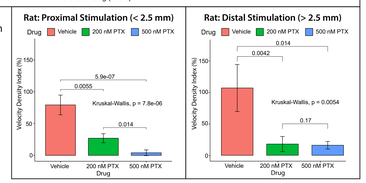


Electrophysiology Over Time: EEA recordings can be completed on the same samples across multiple study days without any observed negative effects, as seen in the example MVP recordings from a human iPSC NerveSim® (**Top Left**). Repeated stimulation of human iPSC NerveSim® plateaued after repeated stimulation (**Top Right**). NerveSim® dosed with vincristine (Vn; **Bottom Left**) show a dosage dependent decrease in responses across time (**Bottom Right**).

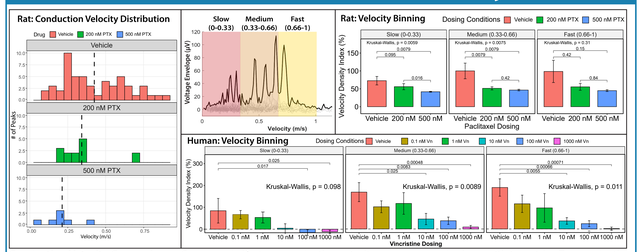
Spatial Responses



Spatial Electrophysiology: MVP traces were separated depending on the location of the stimulation electrode with respect to the spheroid and calculated for 2 conditions: proximal (<2.5 mm) and distal (>2.5 mm) stimulation. Vincristine dosing of human NerveSim® (**Top**) showed an early decline in distal responses while the proximal responses persisted for longer. Paclitaxel dosing in rat NerveSim® (**Right**) showed that 7-day dosing knocked out distal responses while proximal responses were greatly reduced.

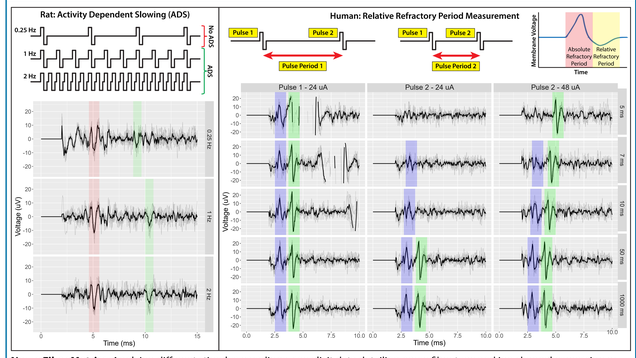


Nerve Conduction Velocity



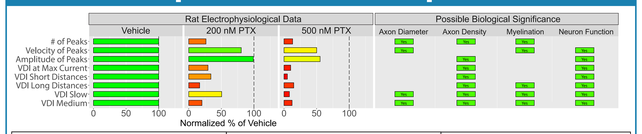
Conduction Velocity Comparisons: Compound effects on conduction velocity were measured using multiple metrics. The conduction velocities of significant peaks in the electrophysiological recordings yielded a distribution in rat NerveSim® samples (**Left**) that decreased with dosing of paclitaxel. Conduction velocity was also measured by binning the MVP signal (**Top Middle**), which showed significant effects for paclitaxel dosing of rat NerveSim® (**Top Right**) and vincristine dosing of human NerveSim® (**Bottom Right**).

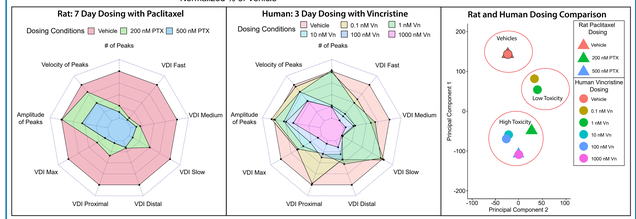
Nerve Fiber Type and Health



Nerve Fiber Metrics: Applying different stimulus paradigms can elicit data detailing nerve fiber type and ion channel expression or health. Increasing the stimulus frequency from low (0.25 Hz) to high (1-2 Hz) causes ADS in C-fibers expressing Nav 1.7 ion channels, as seen in the example for rat NerveSim® (**Left**). A paired pulse stimulation with varying pulse periods (**Right**) can be used to quantify the relative refractory period for neurons, which increases with neuropathy.

Compound Effect Comparison





Compound Effects Summary: Averaging the various electrophysiological metrics across dosing conditions allows characterization of the unique way each drug affects the NerveSim® platform, which corresponds to different biological mechanisms (**Top**). The toxicity profiles for dosing rat NerveSim® with paclitaxel (**Bottom Left**) and human NerveSim® with vincristine (**Bottom Middle**) shows some similarities and differences between the two species and two compounds. The future goal of the NerveSim® platform is to generate a library of responses from known compounds to see patterns on mechanism of action based on the aggregate electrophysiological responses such as the example on the **Bottom Right**.

Conclusions

- **The NerveSim® platform provides collection of data-rich electrophysiological metrics that can provide insights into neurotoxicity, neuroprotection, and neurorehabilitation**
- **Functional electrophysiological measurements provide the same quantitative metrics as clinical electrophysiology**
- **Future screening of multiple compounds with different mechanisms will generate a database for predicting compound mechanisms based on high-throughput electrophysiology**

For more information: