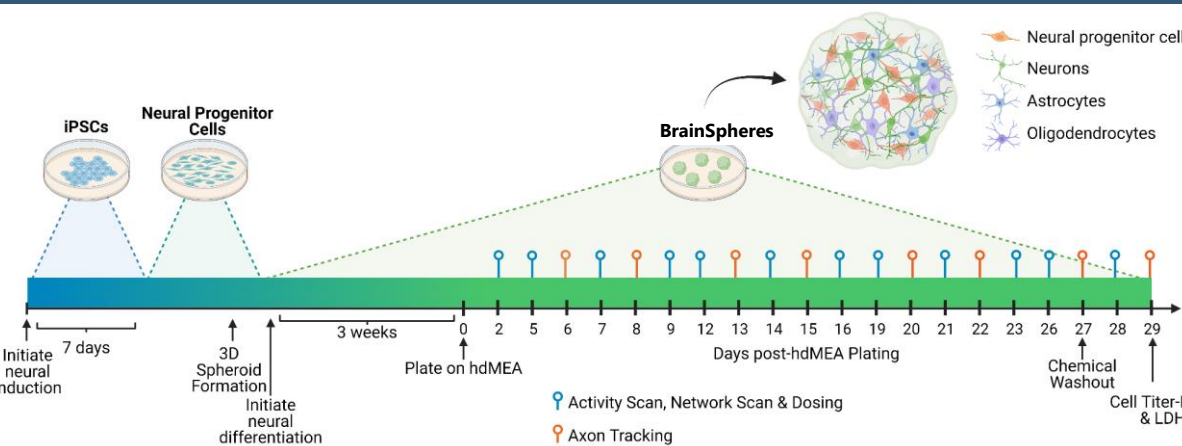


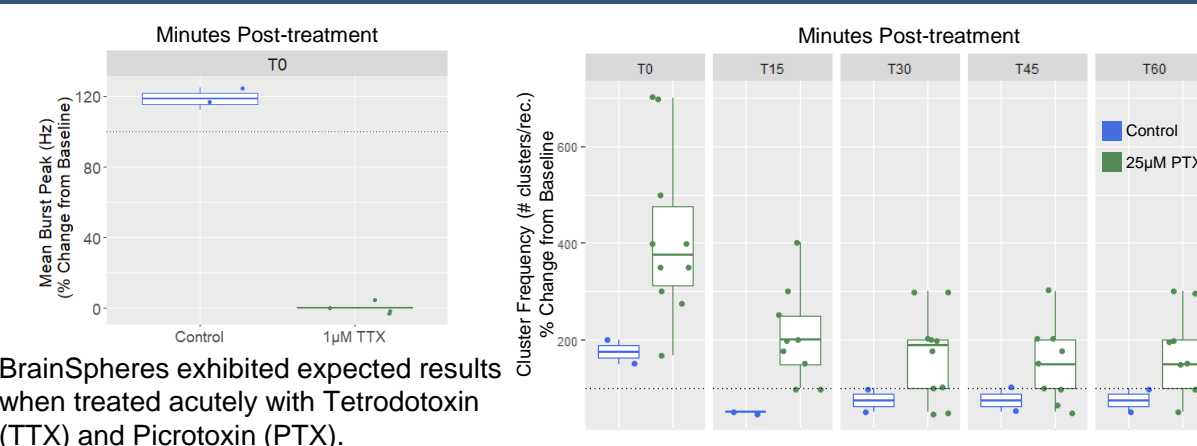
Introduction

- The developing brain is particularly vulnerable to perturbations when exposed to environmental compounds.
- To expand upon the network formation assay (NFA) in the DNT-IVB, we are developing a functional assay utilizing 3D human iPSC-derived neurospheres (BrainSpheres) and high-density Microelectrode Arrays (hdMEAs).

Methods

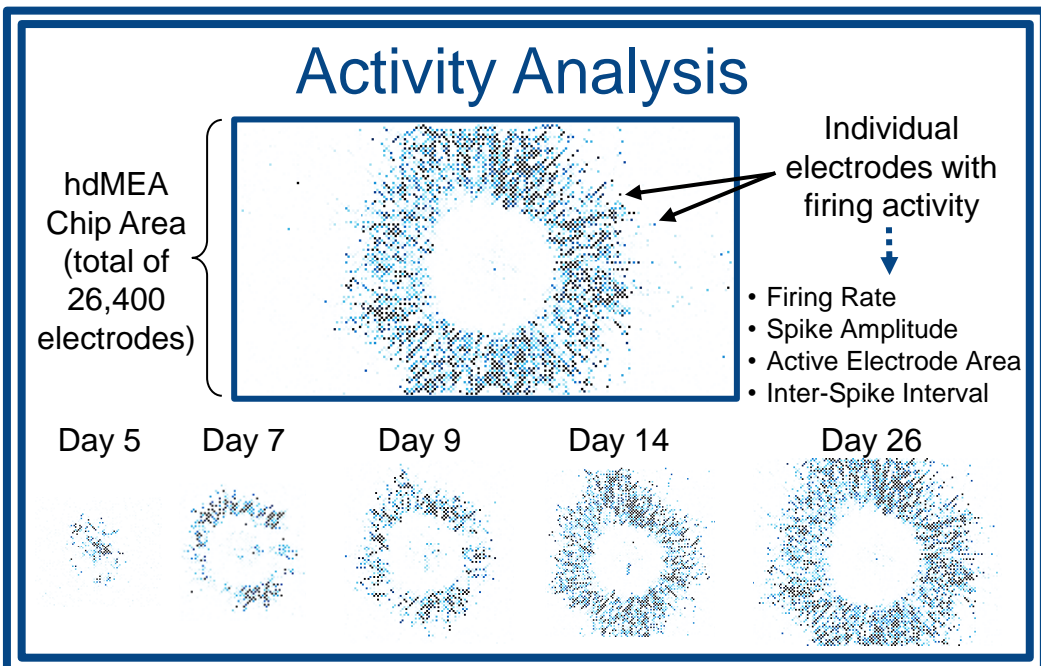


Acute Assay Verification



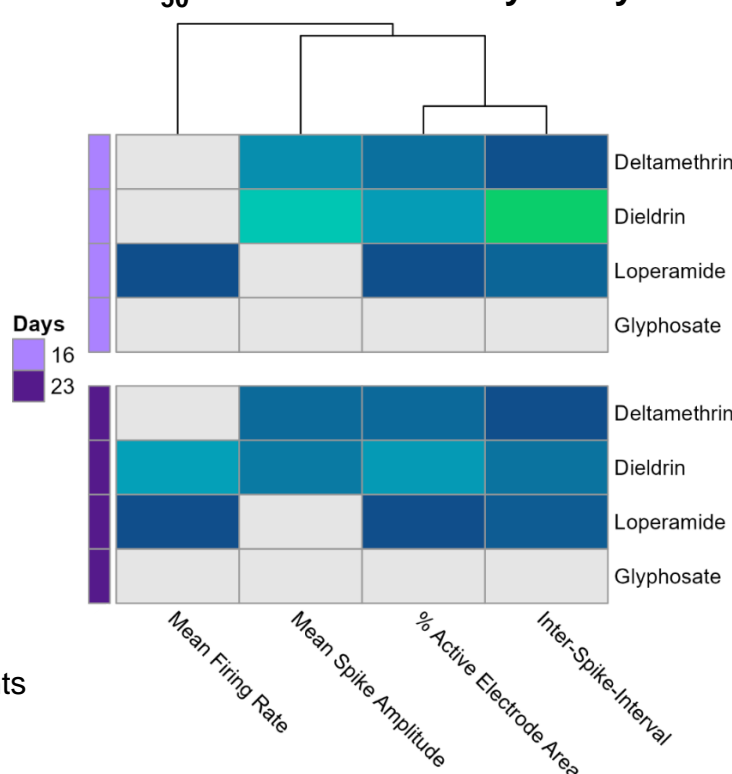
BrainSpheres exhibited expected results when treated acutely with Tetrodotoxin (TTX) and Picrotoxin (PTX).

Deltamethrin, dieldrin, and loperamide exposure in BrainSpheres disrupt neural network size

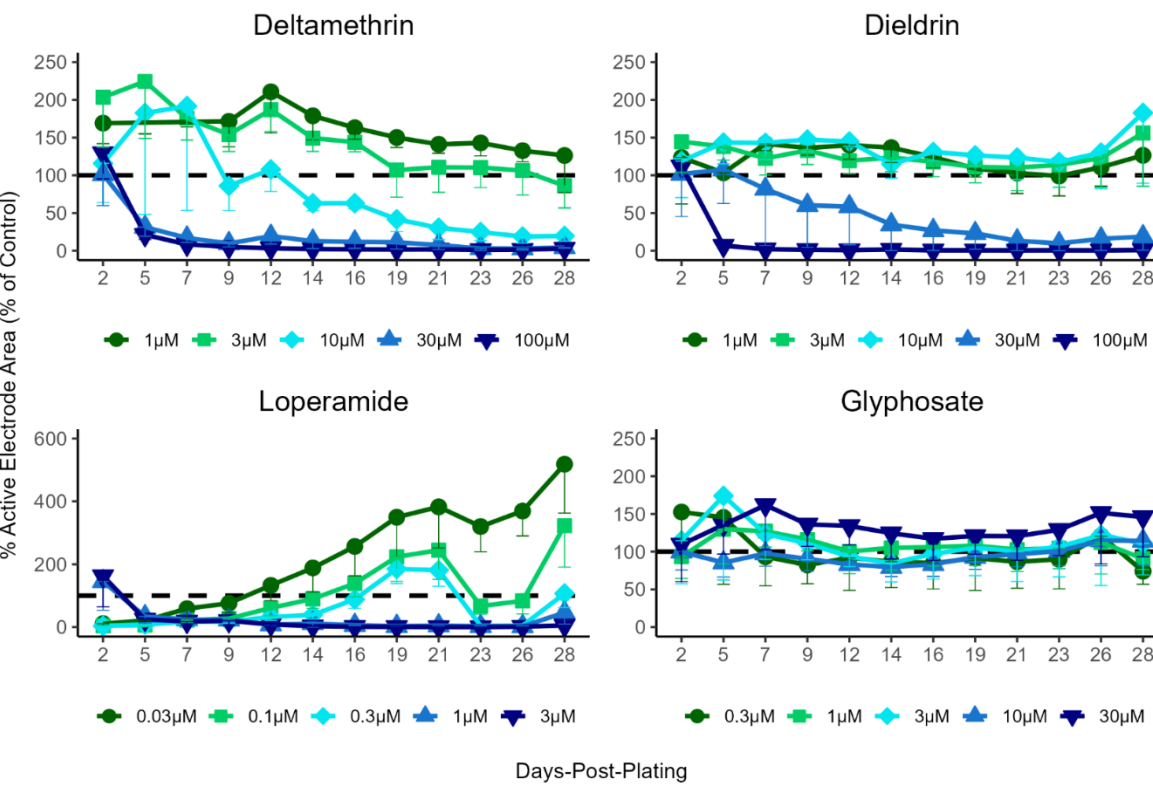


Timepoints where BrainSpheres are developing (Day 16) and reaching maturation (Day 23) showed developmental disruption for several endpoints for all tested chemicals, except glyphosate. Dieldrin and Deltamethrin appeared to have greater effects at Day 23 compared to Day 16.

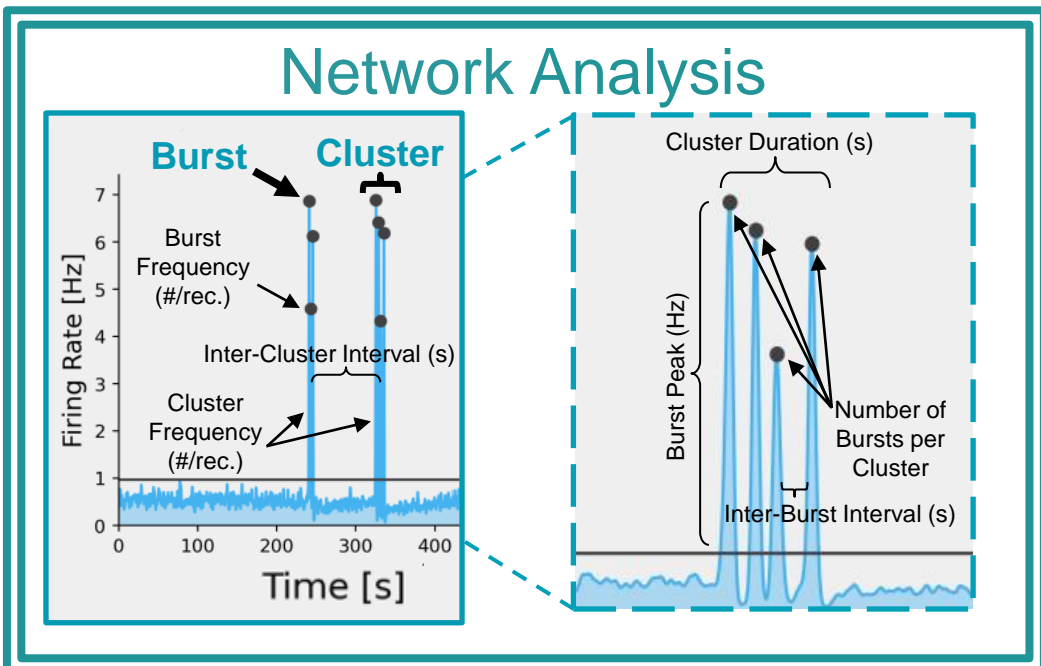
AC₅₀ Values for Activity Analysis



% Active Electrode Area

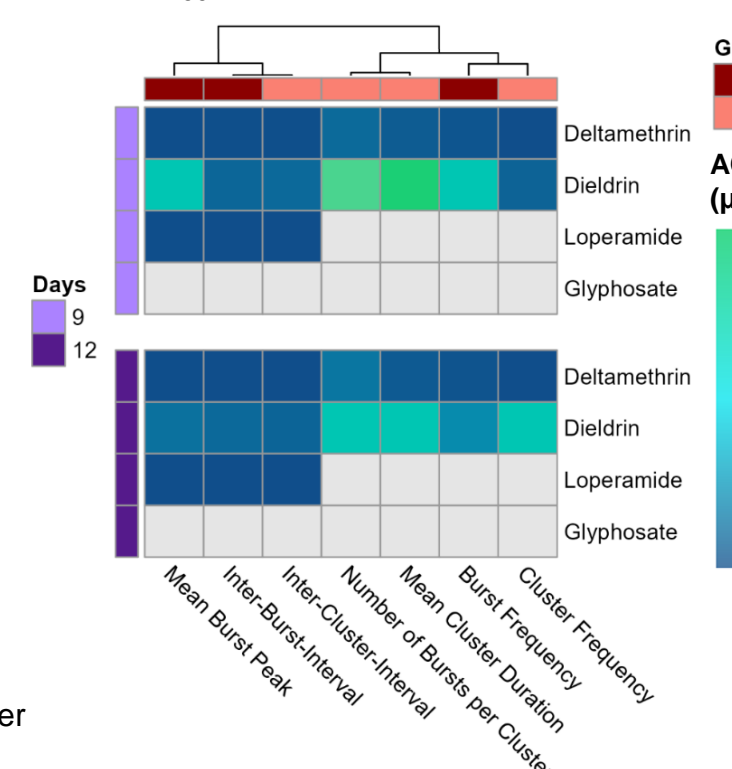


Deltamethrin, dieldrin, and loperamide alter bursting patterns in developing BrainSpheres

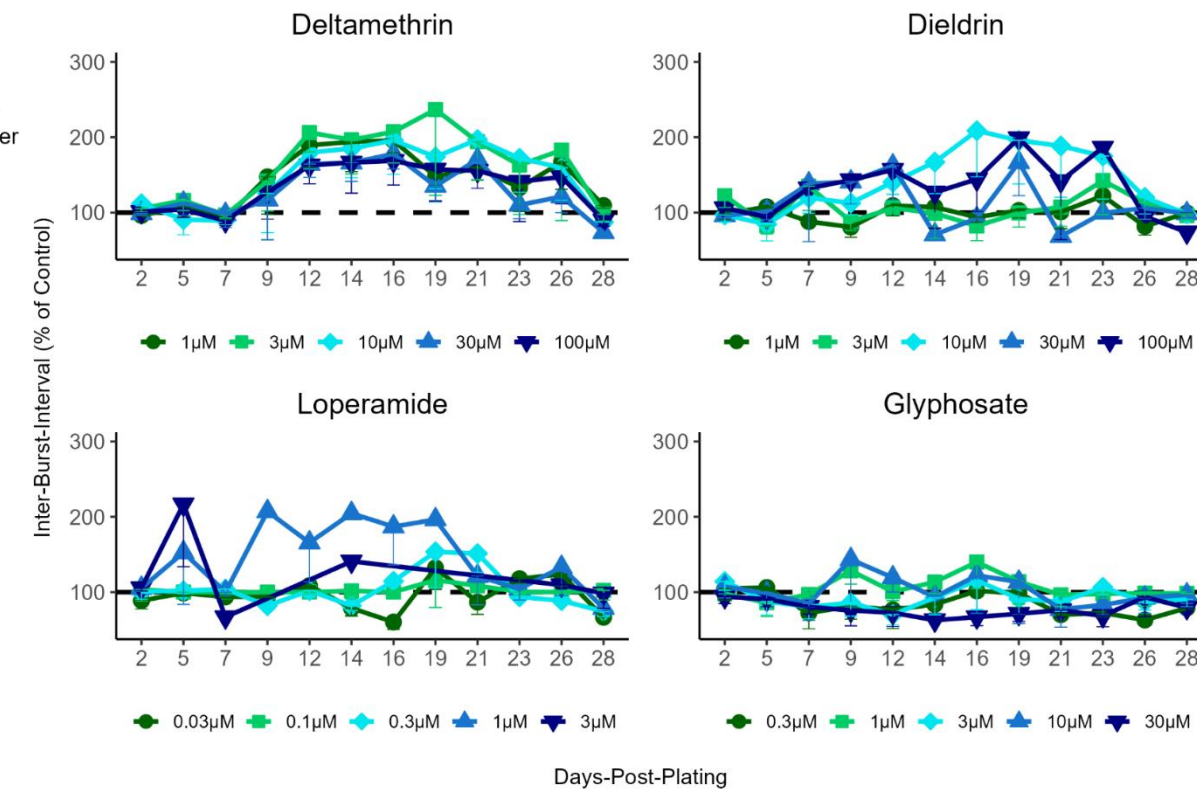


Exposure to deltamethrin, dieldrin and loperamide at timepoints where the neural network in the BrainSpheres are developing (Day 9) and reaching maturation (Day 12) resulted in disruption of network formation, with greater effects seen at Day 12 compared to Day 9. Glyphosate had no impact.

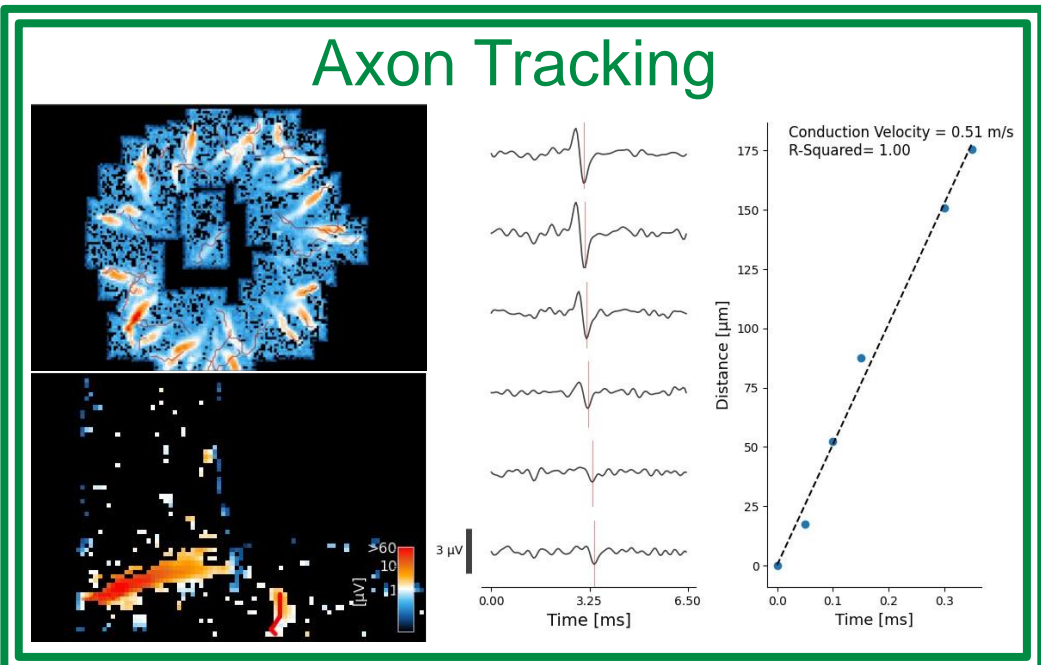
AC₅₀ Values for Network Analysis



Inter-Burst-Interval

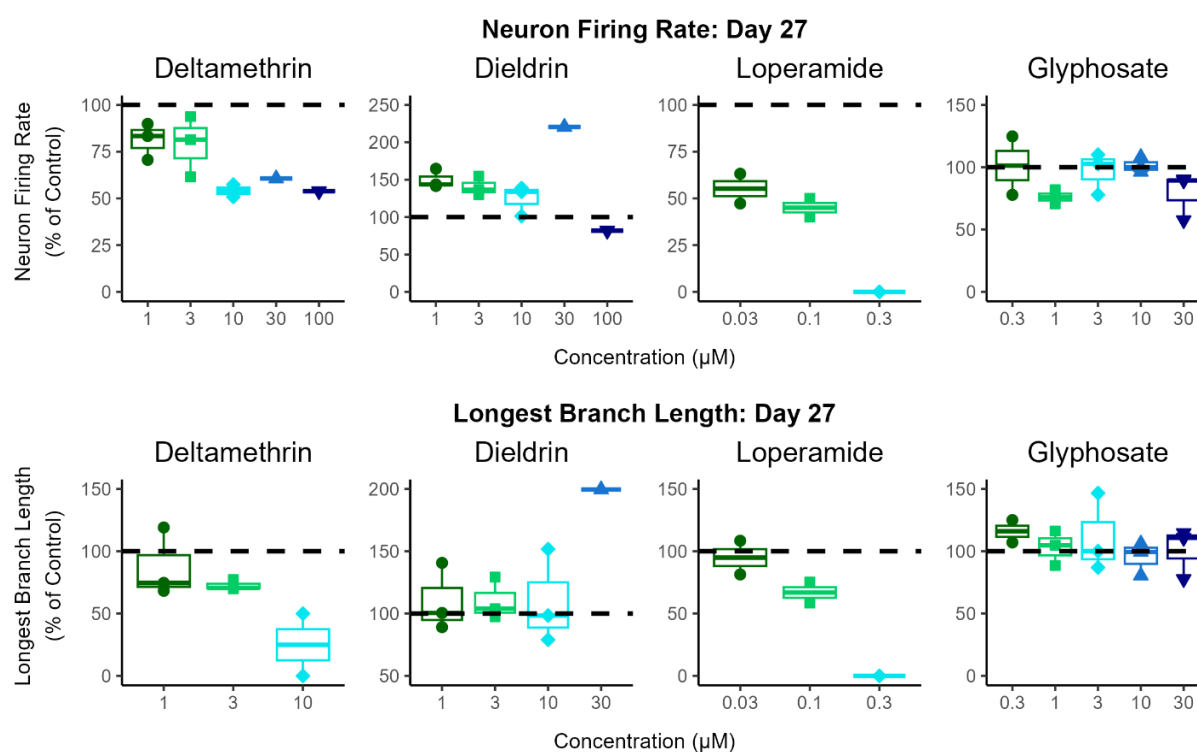
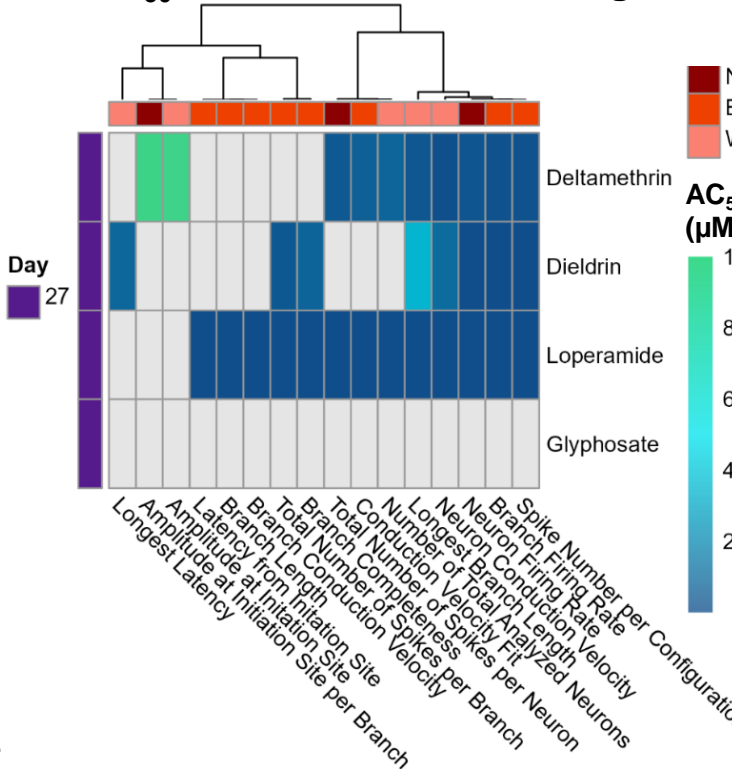


Deltamethrin, dieldrin, and loperamide modify features of action potential propagation



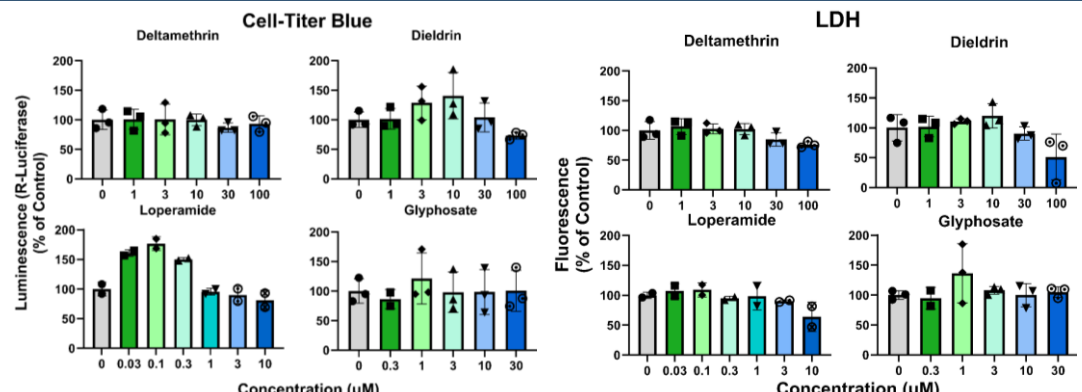
At day 27, when axons are further developed and detectable, several action potential propagation endpoints at the well, branch, and neuron levels are disrupted by exposure to dieldrin, deltamethrin, and loperamide.

AC₅₀ Values for Axon Tracking



Chemical exposure is not cytotoxic to BrainSpheres

Viability assays, Cell-Titer Blue and total LDH, were completed on Day 29. Chemically-exposed BrainSpheres do not show signs of cytotoxicity for deltamethrin, dieldrin, loperamide, nor glyphosate. Statistics were computed using a one-tailed t-test, $p < 0.05$.



Conclusions

- Dieldrin, deltamethrin and loperamide disrupt network formation in human neurospheres without cytotoxicity similar to the rat NFA.
- This BrainSphere-based functional assay could be a valuable addition the DNT-IVB.
- Future studies will expand testing of environmentally relevant compounds, including neonicotinoid insecticides and per- and polyfluoroalkyl substances (PFAS), to demonstrate further the capability of this approach for DNT screening.