

New Approach Methodology (NAM) using 3D Human iPSC-Derived Neural Organoids to Screen for Developmental Neurotoxicity Hazard

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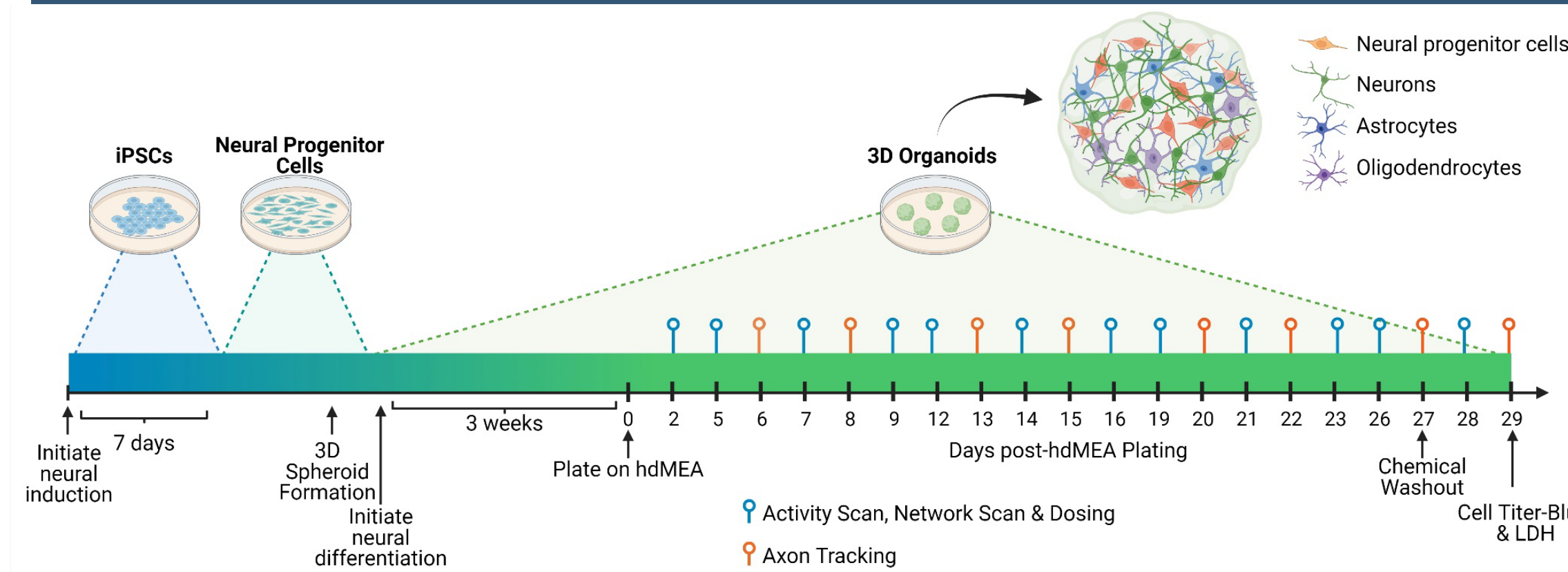
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Introduction

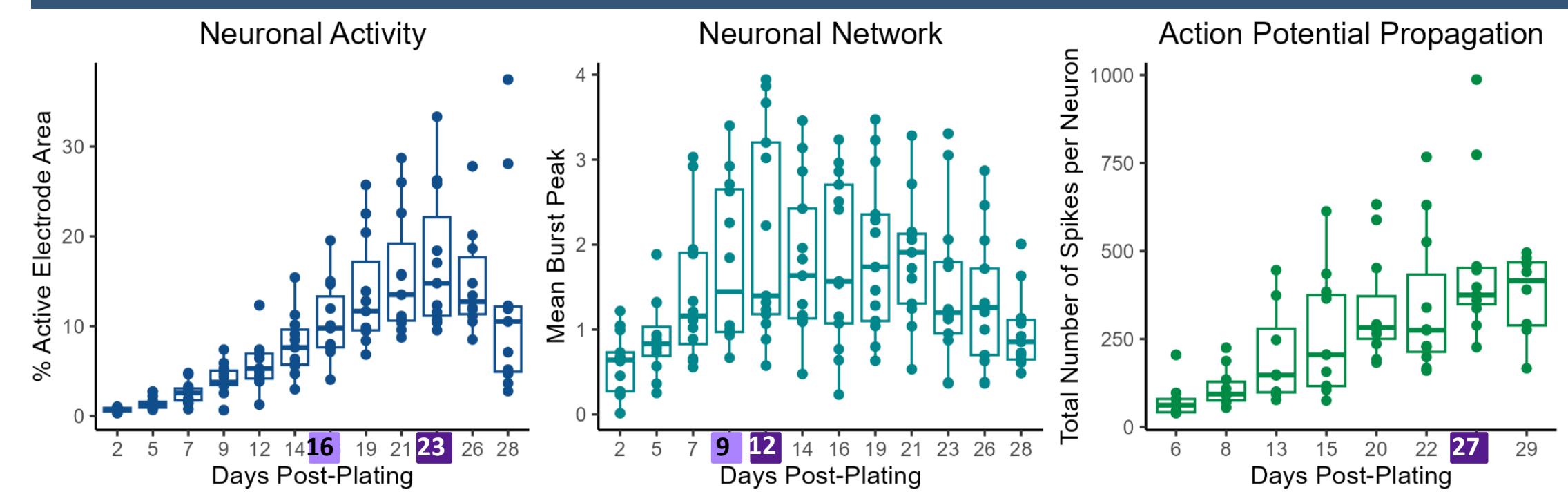
- New approach methodologies (NAMs) have been developed to better recapitulate human biology, increase throughput, lower costs, and reduce the time required for toxicological testing.
- The developing brain is particularly vulnerable to perturbations as a result of chemical exposures; exposures during neurodevelopment can result in developmental neurotoxicity (DNT).
- Current DNT NAMs use 2-dimensional primary rat cortical neural cultures on microelectrode arrays (MEAs) to determine the effects of developmental chemical exposure on neural network formation.
- Here, a 3D human iPSC-derived organoid model is used to screen 4 chemicals (dieldrin, deltamethrin, loperamide, and glyphosate) using high-density MEAs (hdMEAs).

Methods



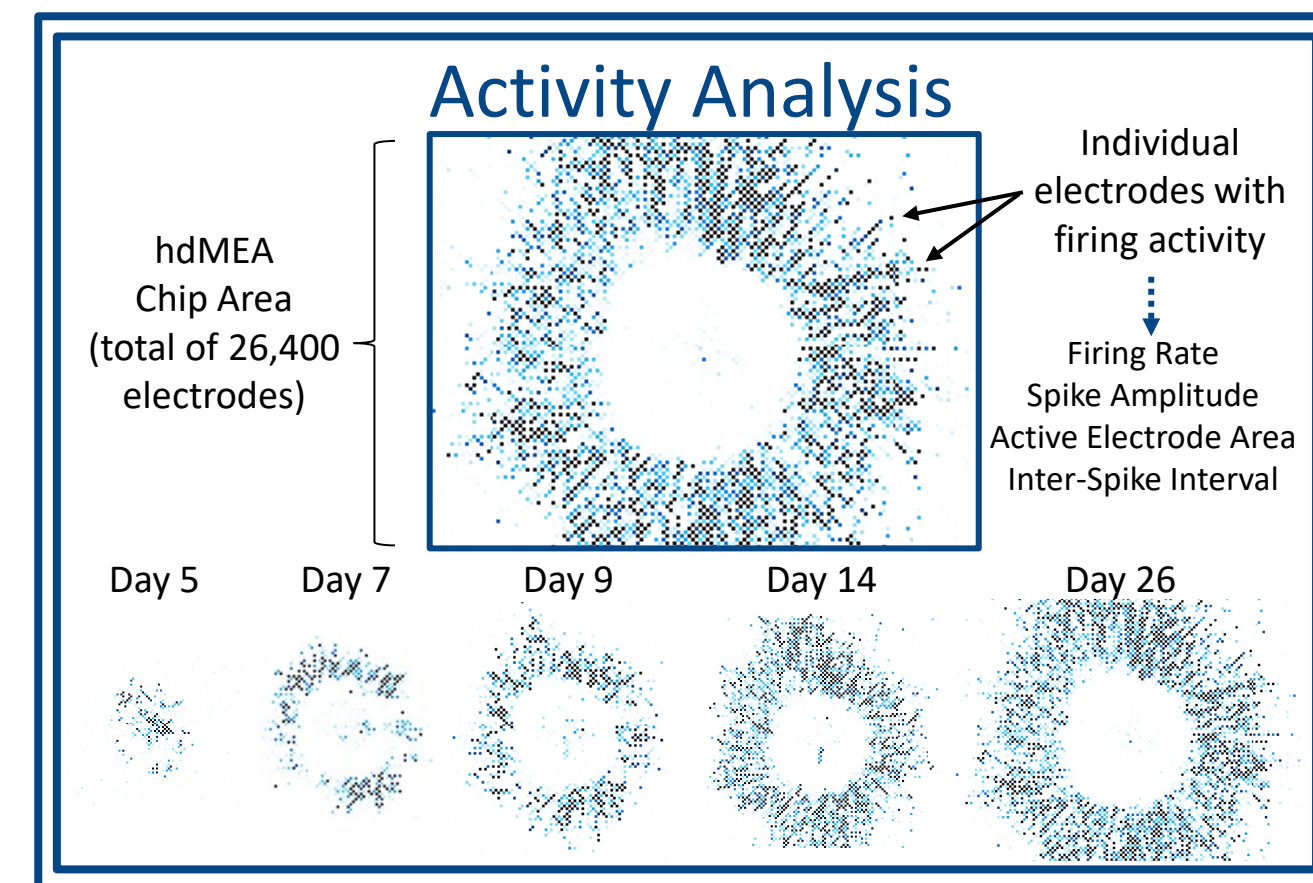
3-week organoids were plated on hdMEAs and treated with chemicals three times a week for 29 days. General activity, neuronal network activity, and features of action potential propagation were recorded as indicated above. Data from chemically treated spheroids at select days were fit to a curve using *tcplfit2*. AC_{50} (concentration at which 50% of maximum activity is observed) was calculated only for active chemicals per endpoint.

Ontogeny of spontaneous activity in neural organoids

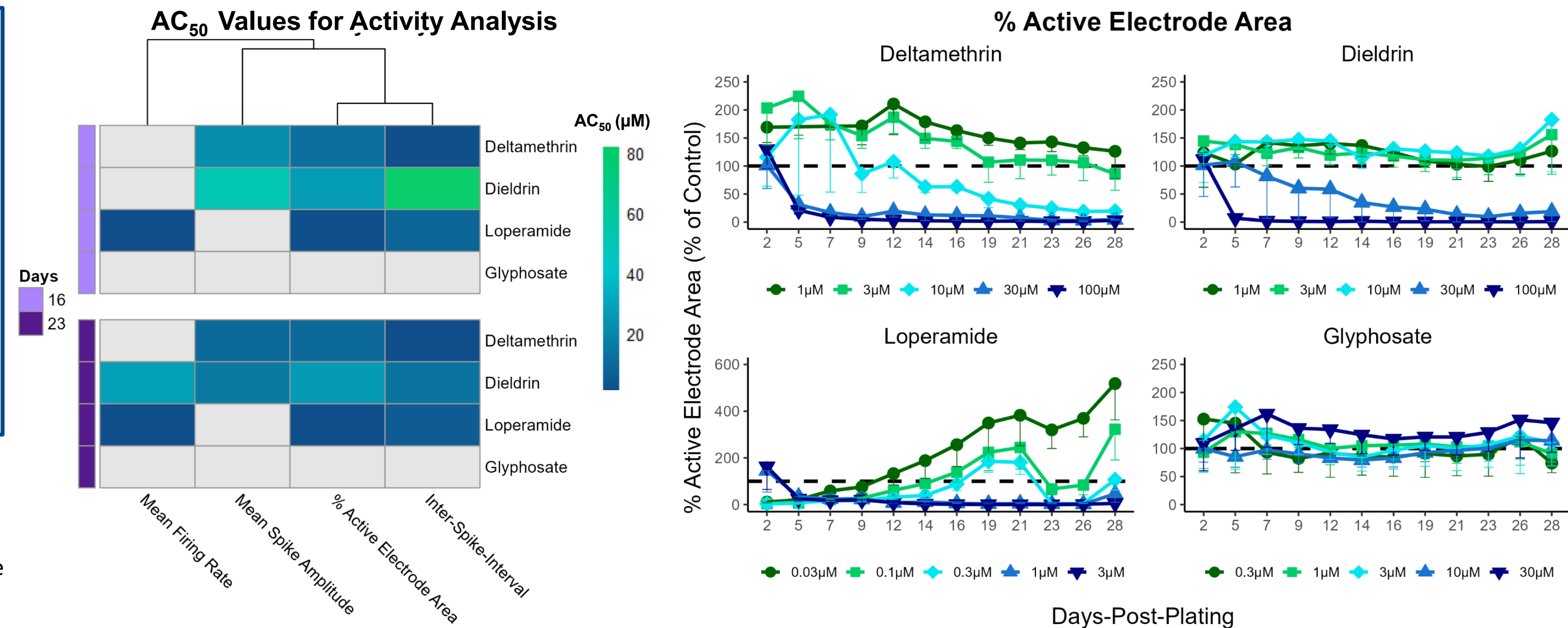


Activity analysis (% of active electrodes), network analysis (mean burst peak), and axon tracking (total number of spikes per neuron) shows an increase in activity of 3-week organoids plated on hdMEAs for up to 29 days. The increase in activity is an indication of neurodevelopment and neural network formation. Organoid network formation appears to develop earlier and plateaus at day 12 while general activity plateaus near day 23. Axonal firing and axon branch arborization is the last phase of organoid development reaching maturation at Day 27. The boxes around select days indicate the days where AC_{50} values were calculated in the following heatmaps.

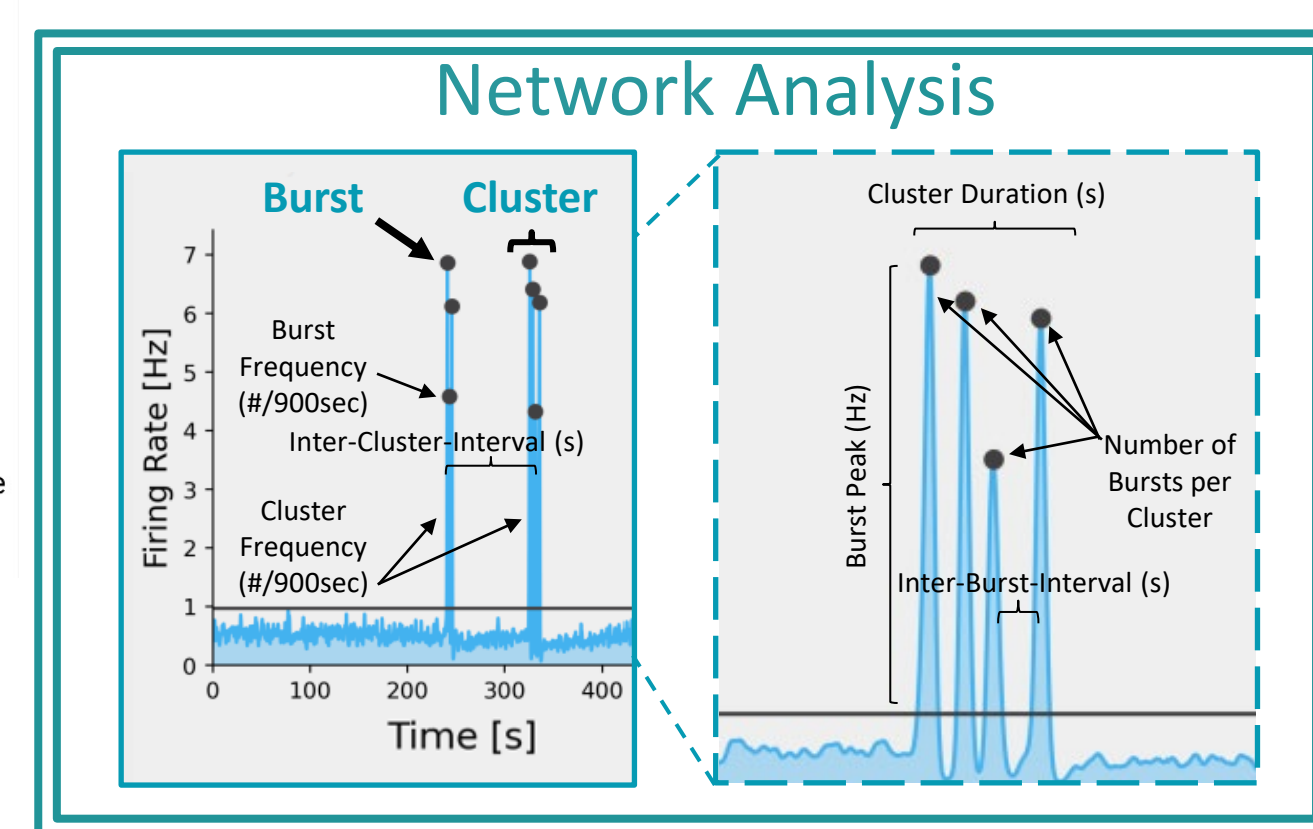
Deltamethrin, dieldrin, and loperamide exposure in organoids disrupts neuronal network size



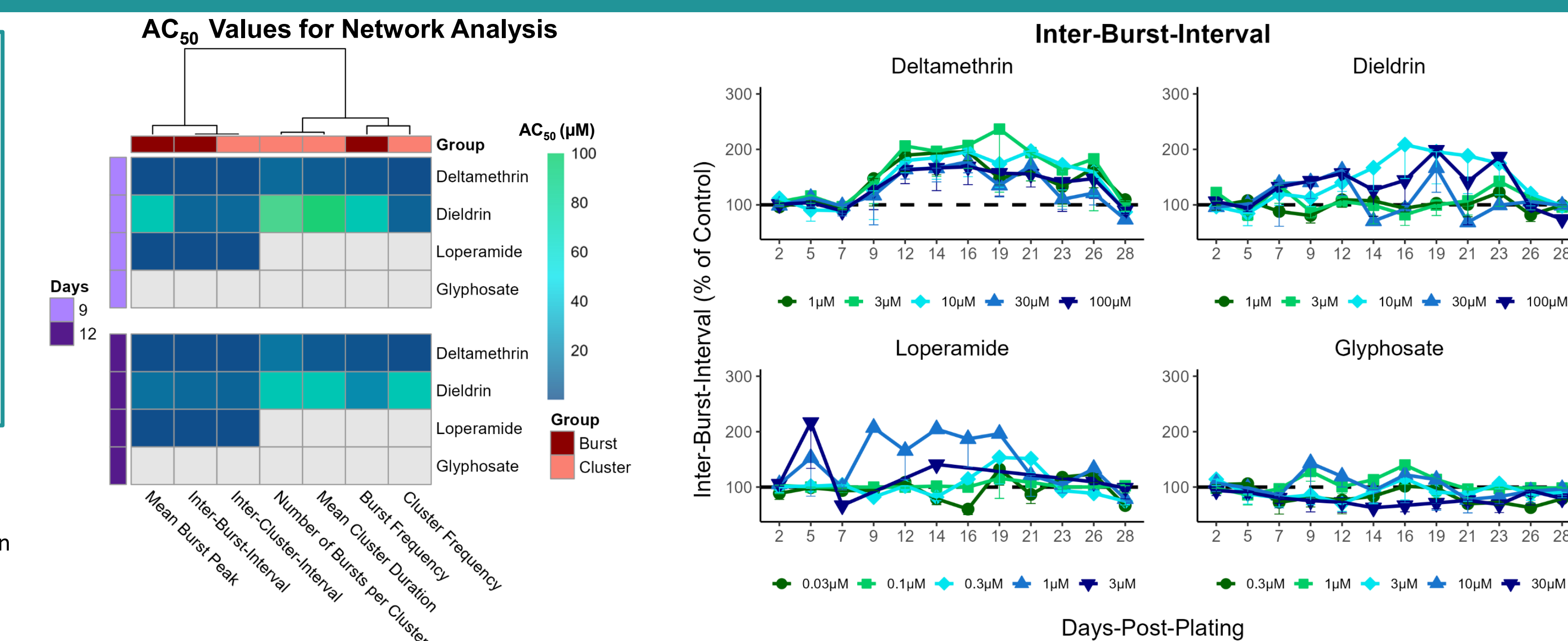
Timepoints where organoids are developing (Day 16) and reaching maturation (Day 23) show developmental disruption for several endpoints for all tested chemicals, expect glyphosate. Dieldrin and Deltamethrin appear to have greater effects at Day 23 compared to Day 16.



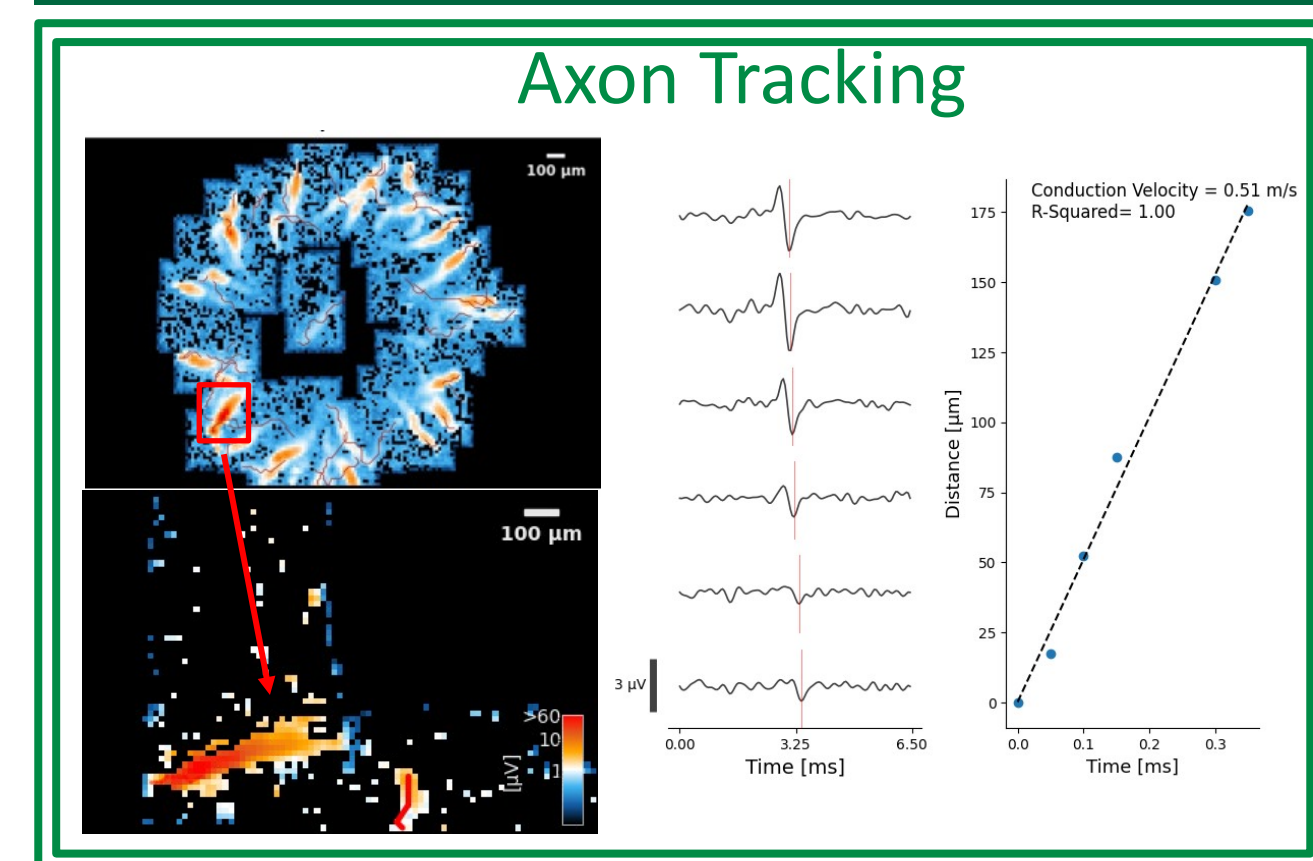
Deltamethrin, dieldrin, and loperamide alter bursting patterns in developing neural organoids



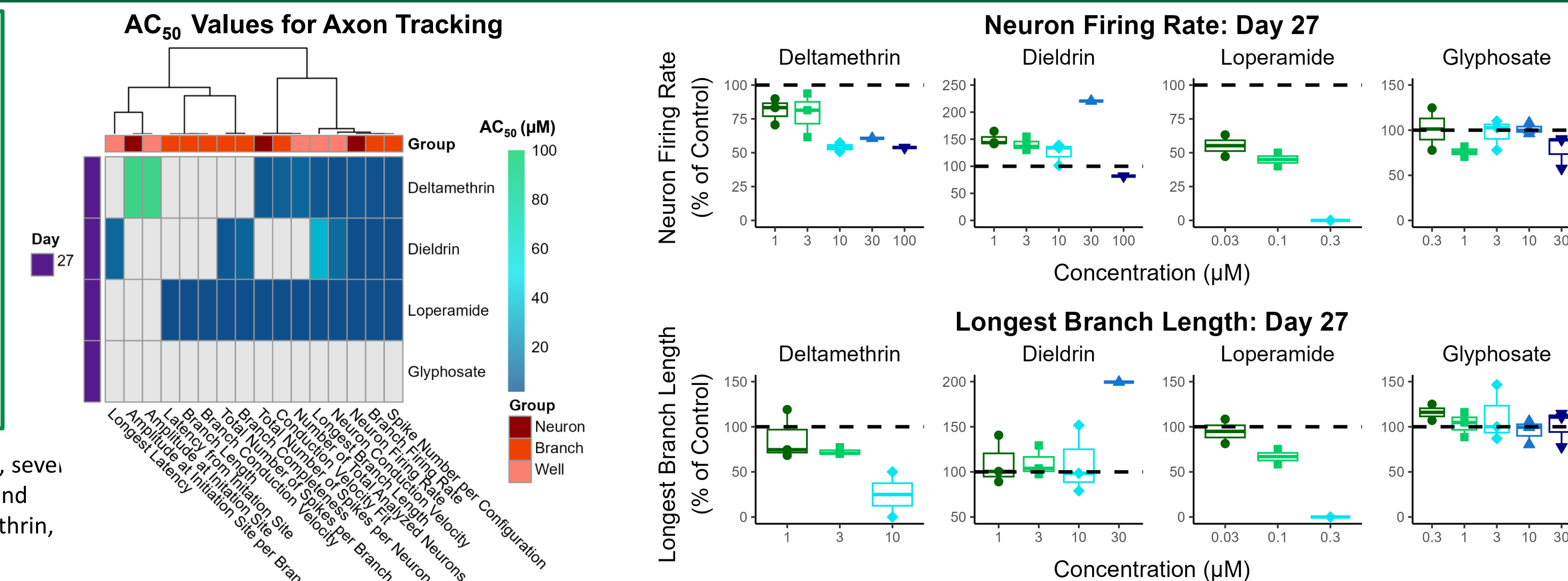
Exposure to deltamethrin, dieldrin and loperamide at timepoints where neural network in organoids is developing (Day 9) and reaching maturation (Day 12) results in disruption of network formation, with greater effects seen at Day 12 compared to Day 9.



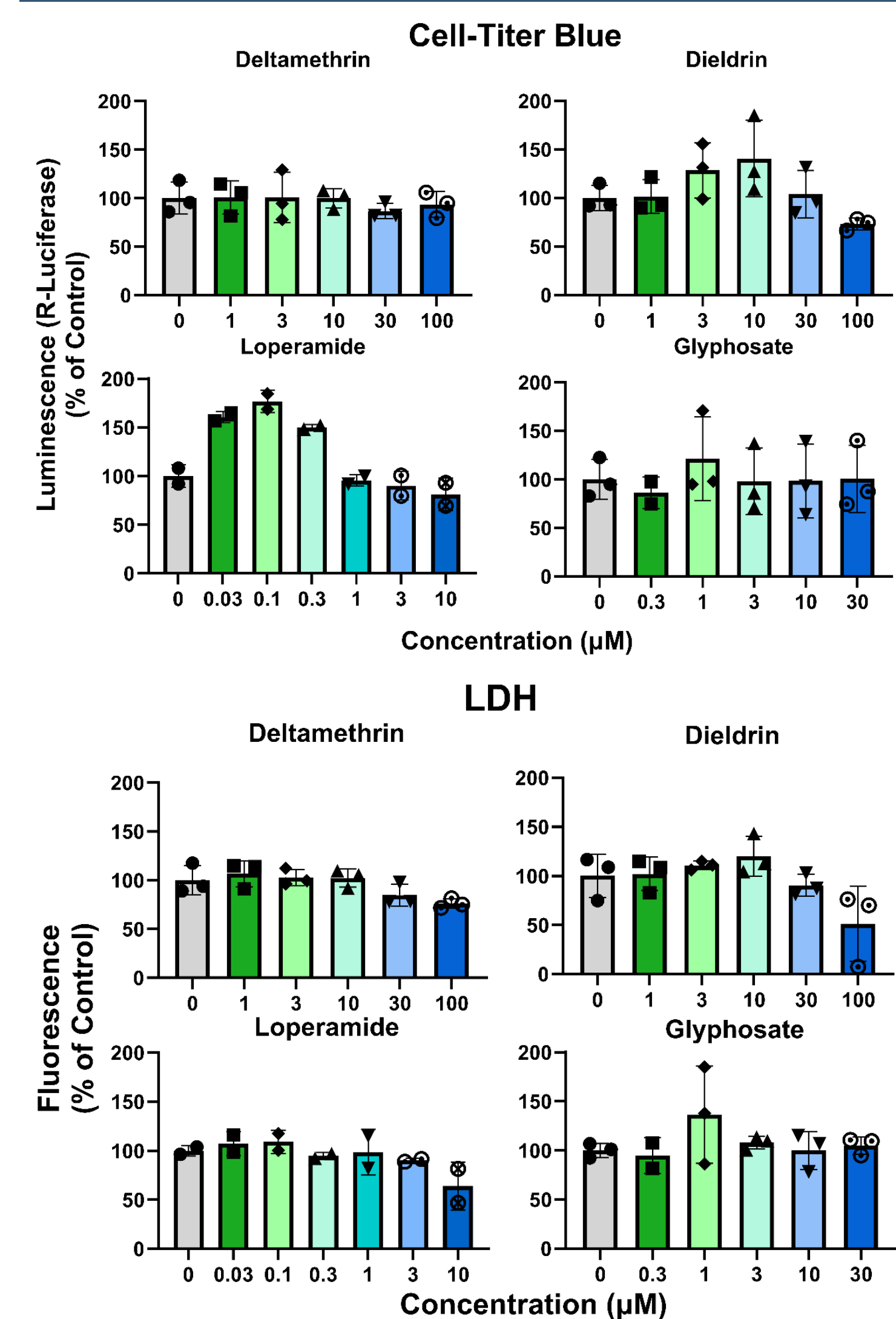
Deltamethrin, dieldrin, and loperamide modify features of action potential propagation



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



Chemical exposure is not cytotoxic



Chemical exposure to deltamethrin, dieldrin, loperamide and glyphosate do not show signs of cytotoxicity (Cell-Titer Blue and total LDH). Statistics were computed using a one-tailed t-test, $p < 0.05$.

Conclusions

- Preliminary data demonstrates that loperamide, dieldrin, and deltamethrin exposure to 3-week organoids alters multiple metrics of network formation.
- The chemical-induced disruption in neuronal activity was selective, occurring without inducing overt cytotoxicity in the organoids.
- These results are concordant with effects of these chemicals in rat 2-dimensional cortical cultures on MEAs.
- Future experiments will expand on preliminary results and test additional chemicals, including PFAS compounds.

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