

Using MEAs to screen chemicals for potential neurotoxicity and developmental neurotoxicity

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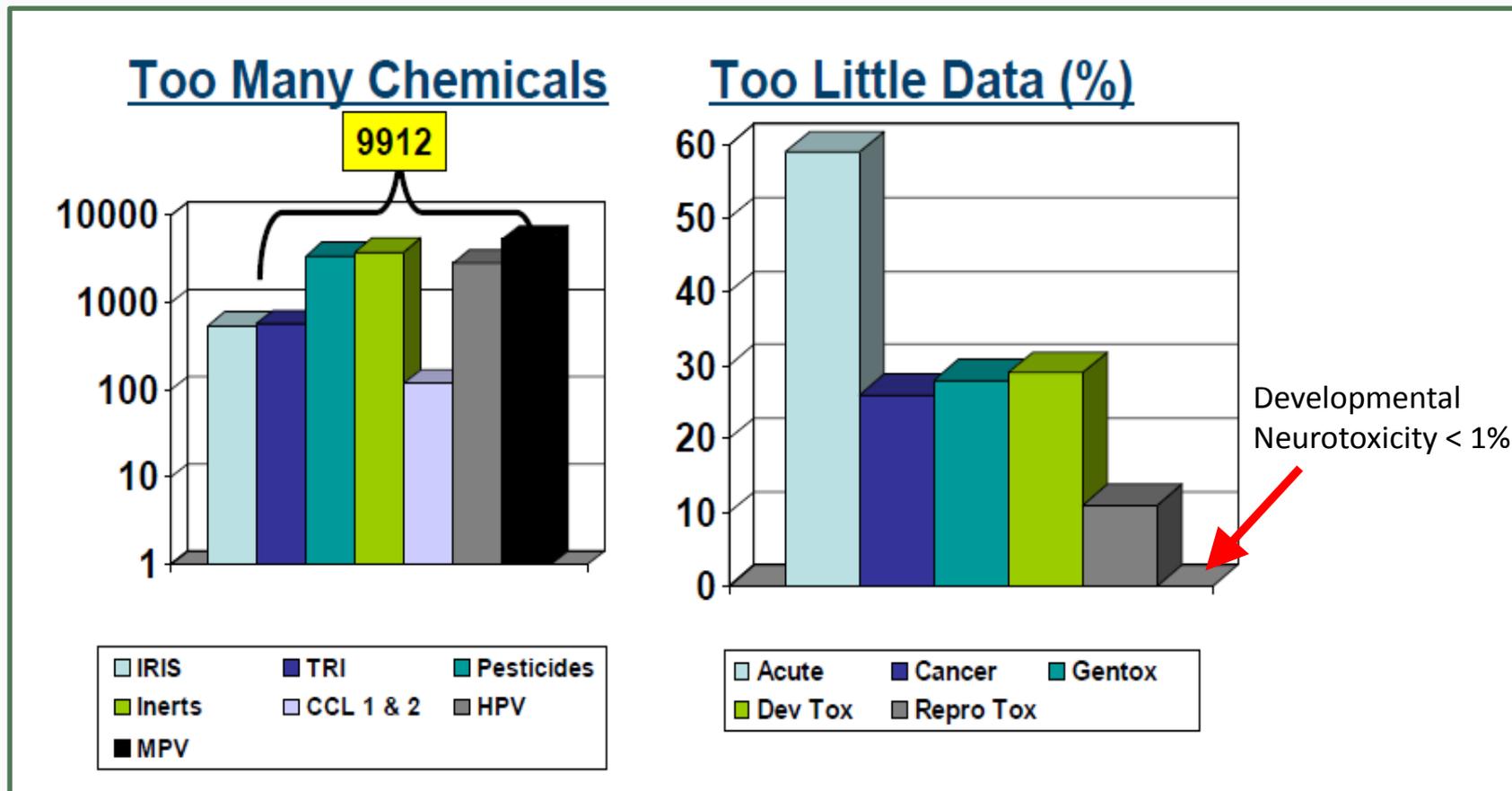
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Outline

- Introduction
- Background on Microelectrode Array (MEA) recording
- Screening Toxcast compounds for acute effects on neural network function
- Screening DNT reference compounds to develop an MEA-based network development assay

*This research is still in progress, and is not yet published.

The problem of too many chemicals.. not enough resources...



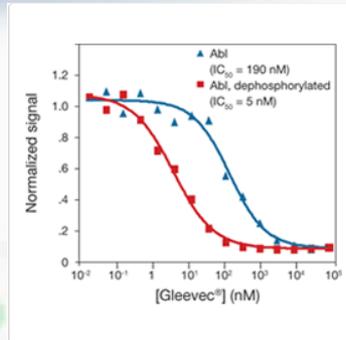
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Methods for in vitro Neurotoxicity assessment

Biochemical Endpoints

(e.g. ToxCast)

- ion channels
- AChE
- thyroid hormone metabolism
- growth factor receptors
- cell adhesion molecules



Structure

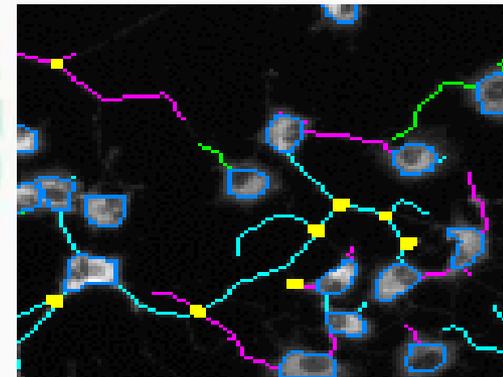
Morphological Endpoints

- Neurite outgrowth
- Cell type
- Synapse number
- Proliferation

Functional Endpoints

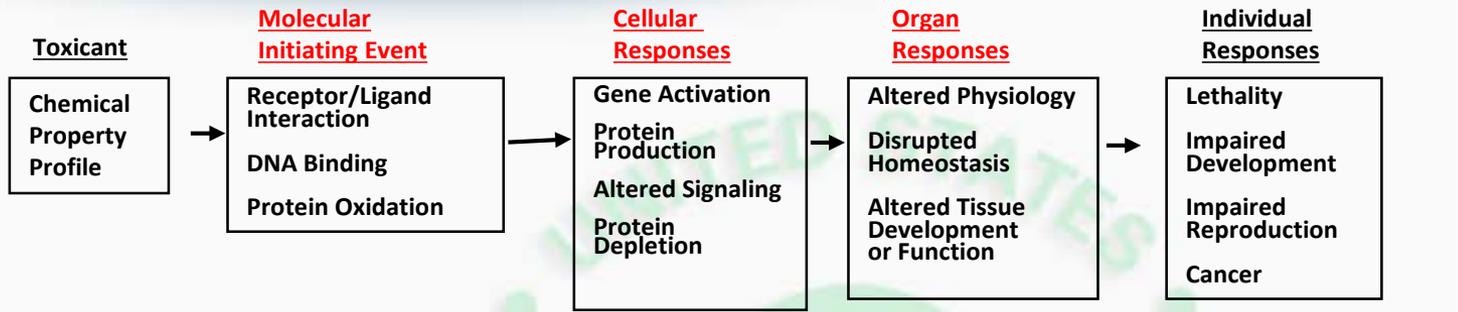
- Patch clamp electrophysiology
- Ion homeostasis (e.g. Calcium imaging)
- Membrane potential
- Mitochondrial Function
- Microelectrode array (MEA) recording

Individual Cell



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Adverse Outcome Pathways (AOPs) for Neurotoxicity Screening



Initiating Event
? (many)

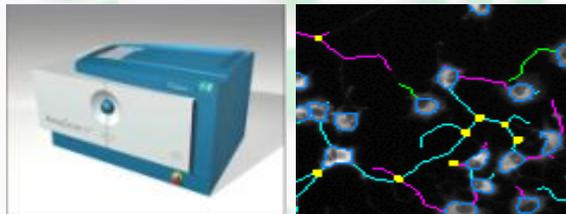
Key Events
differentiation
neurite growth
Synaptogenesis
...

Nervous System
↓ connectivity

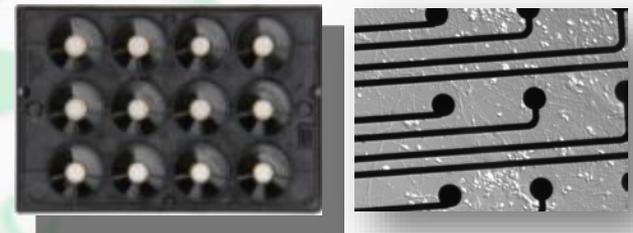
Adverse Outcome
cognitive deficit

Biochemical assays
(e.g. ToxCast)
ion channels
AChE
thyroid hormone metabolism
growth factor receptors
cell adhesion molecules
...

High-Content Screening (HCS)



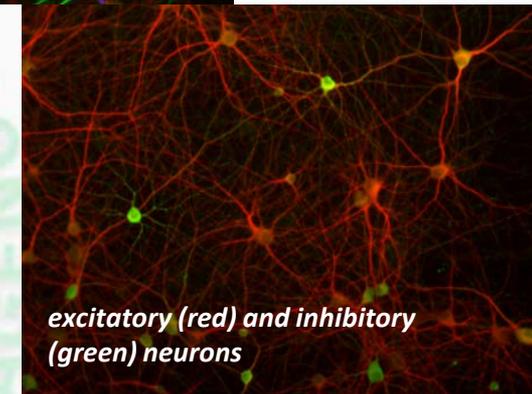
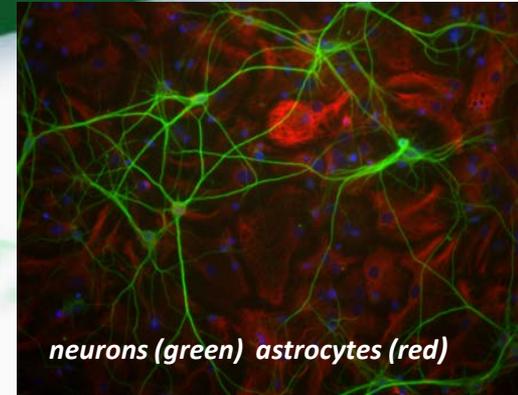
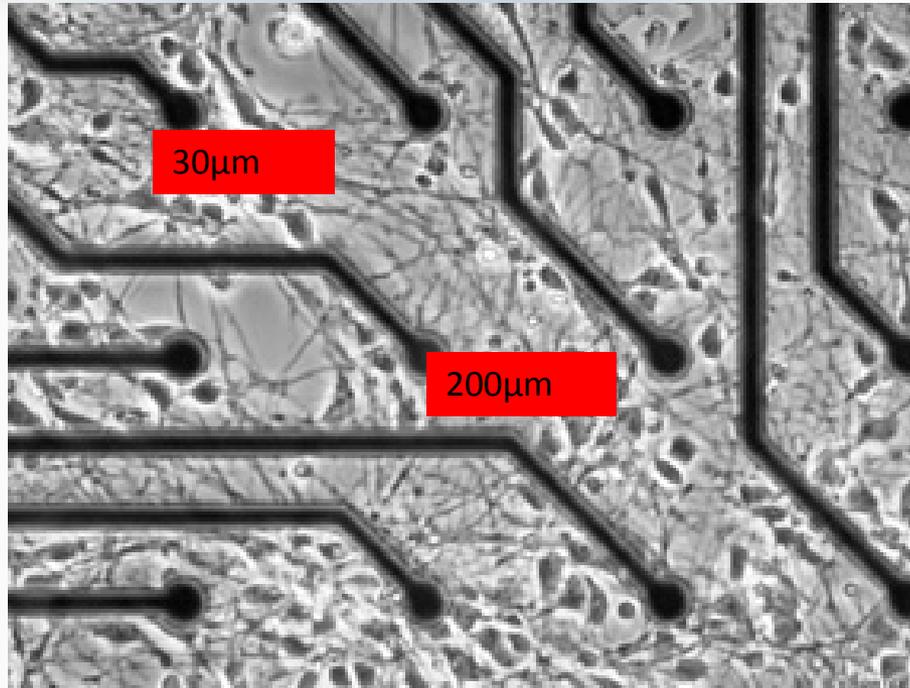
Micro-Electrode Array (MEA)



Increasing Throughput

Increasing Biological Complexity

Introduction to Microelectrode Array (MEA) Recording



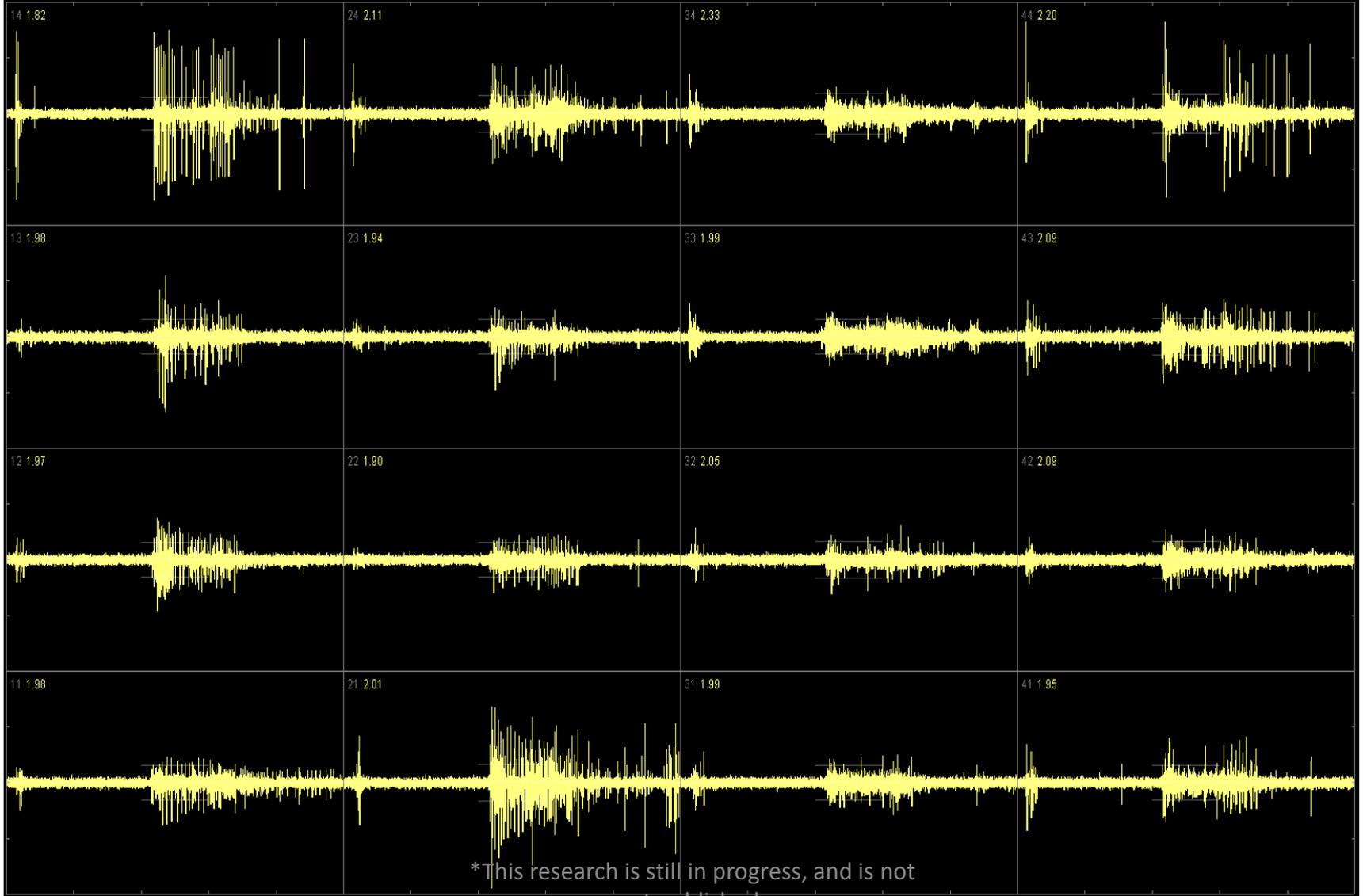
- Array of extracellular electrodes
- Each electrode can record event rates and patterns of any tissue in “contact” with it.
- **Spontaneous** or Evoked Activity

- **Cortical Neurons**

- Hippocampal Neurons
- Spinal cord Neurons
- Retinal neurons
- Retina
- Slices

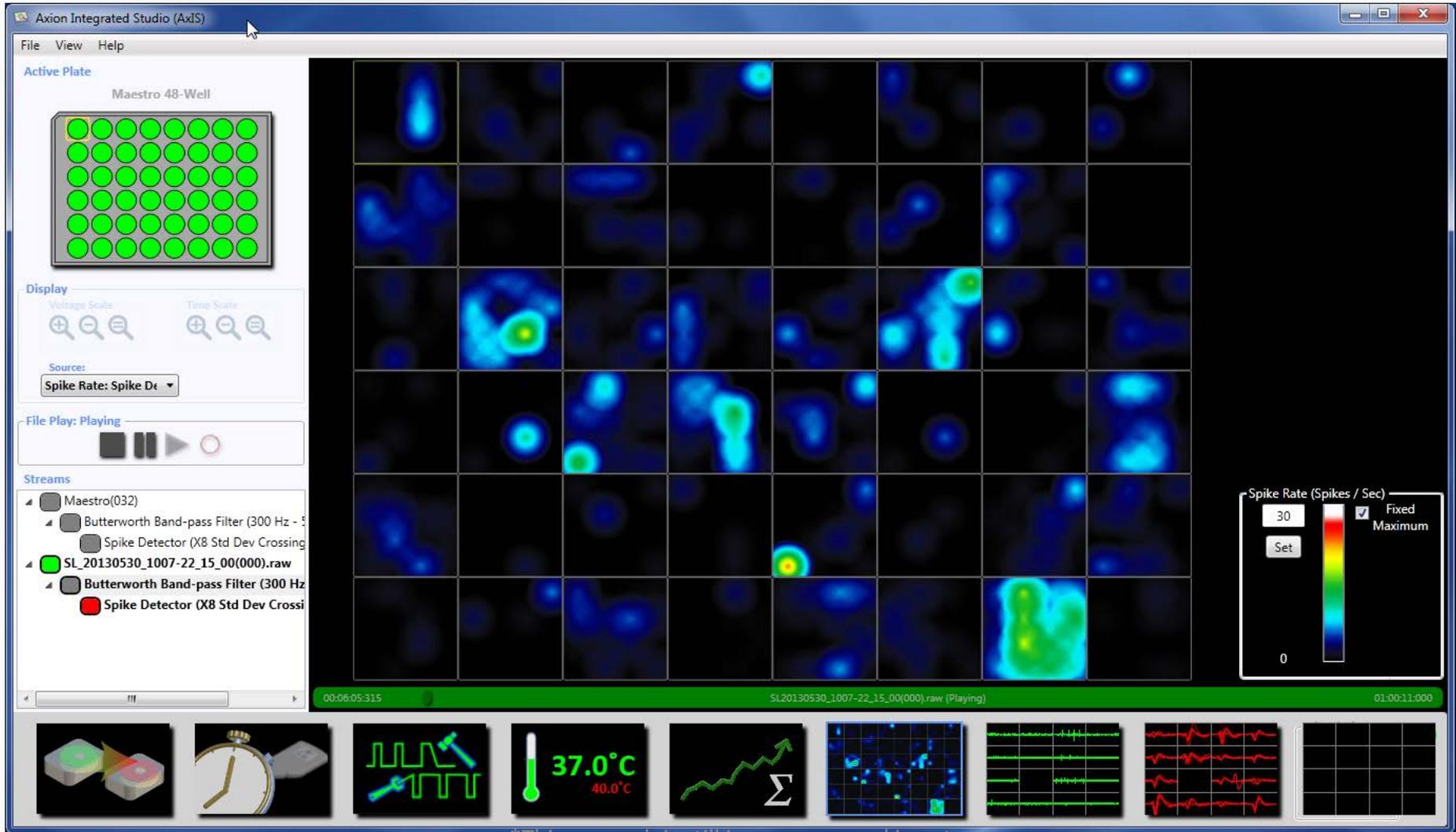
- **Primary Cardiac Cells**

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Cortical culture + MEA = “Brain-on-a-Chip”



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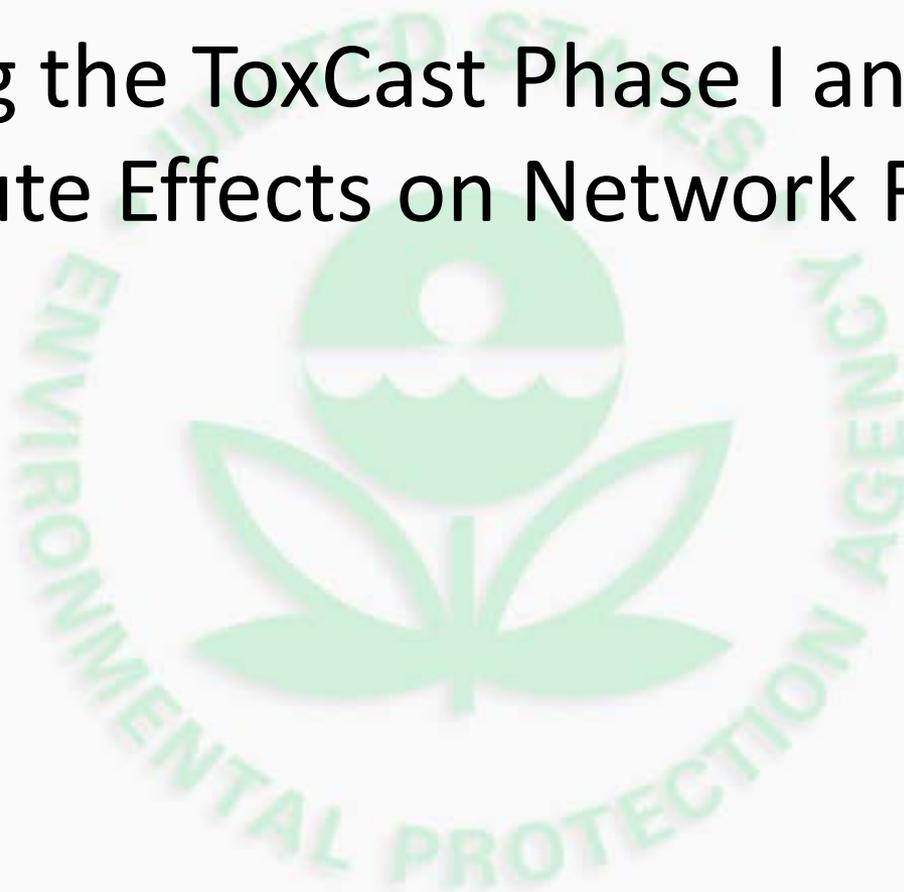
Advantages of MEAs for Neurotoxicity Screening

- Phenotypic, cell-based screen.
- Apical- integrates the responses of multiple ion channels, kinases, receptors, etc
- High Content- Rich temporal and spatial information
- Amenable to pharmacological manipulations
- Can be applied to neurons from multiple brain regions
- Non-invasive
- Complex culture system (multiple neuronal types + glia)
- Repeated measurements over time

The throughput of multiwell plates is sufficient for neurotoxicity screening.

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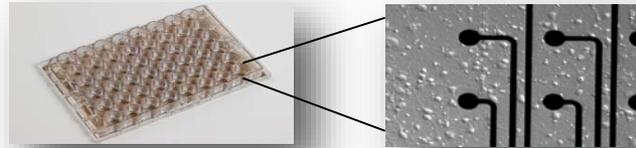
Screening the ToxCast Phase I and II Library for Acute Effects on Network Function



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Multiplexed Experimental Design

Determine Effects on Spontaneous Network Activity



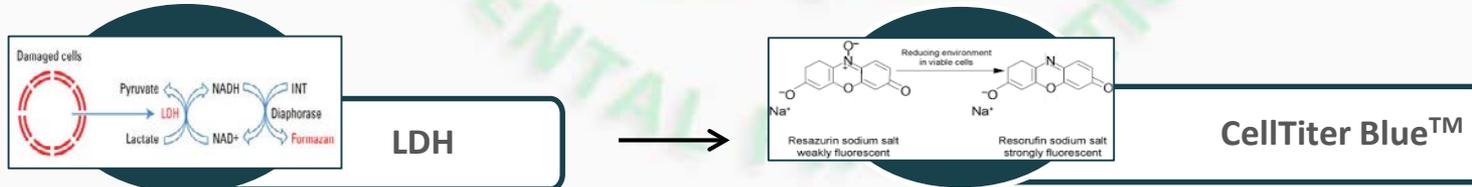
1. Primary cortical neurons are cultured in 48 well MEA plates and allowed to mature for 13 days



2. Plates are placed in the Axion Maestro MEA amplifier

3. Determine firing rate in each well for 40 min prior to and after treatment with compounds

Determine Effects on Cell Health



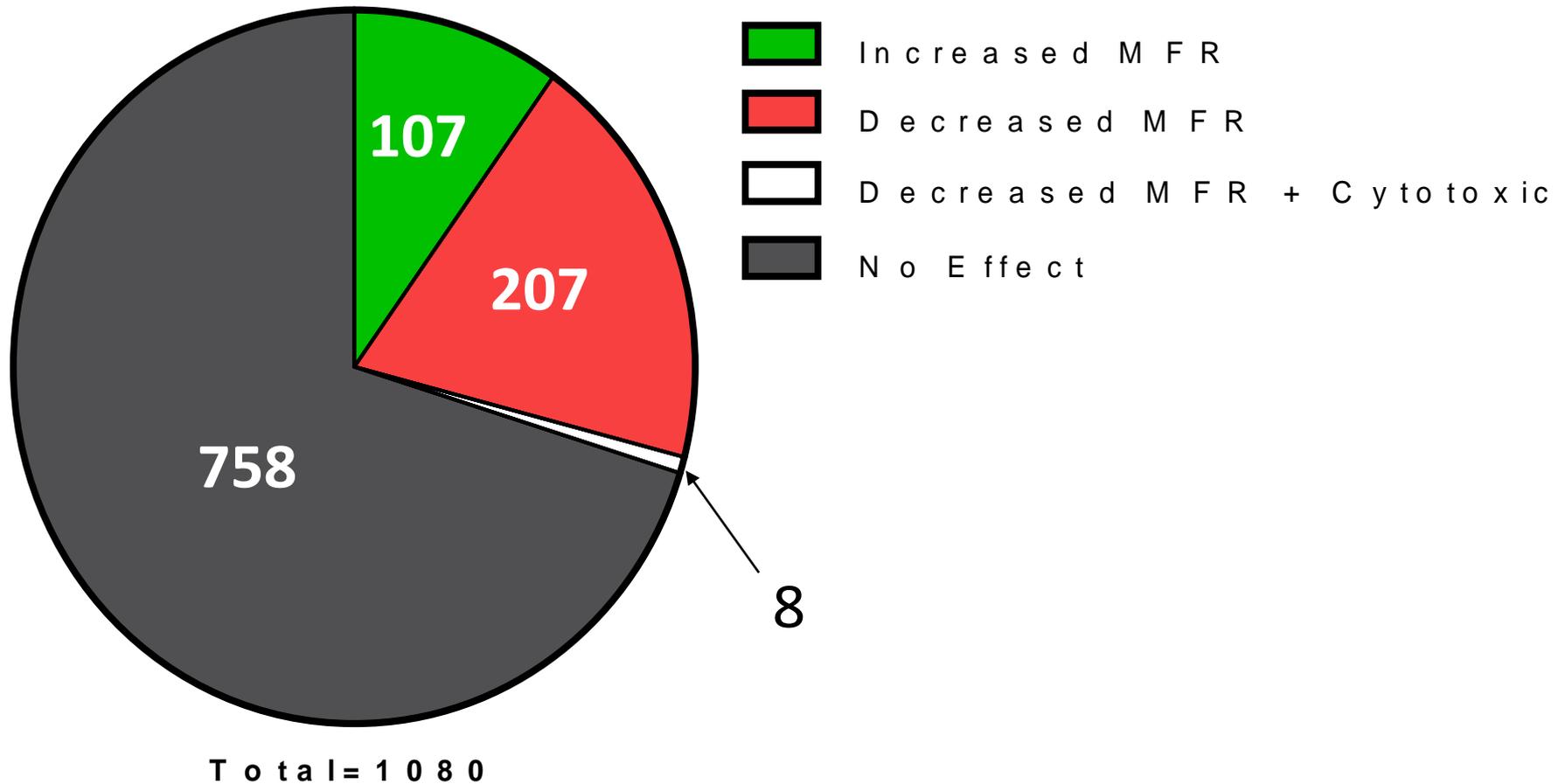
4. Transfer 50 μ L of media from mw MEA to 96 well assay plate.

5. Replace media with 200 μ L of fresh media containing CellTiter Blue™ reagent, incubate for 1 h at 37° C, then read.

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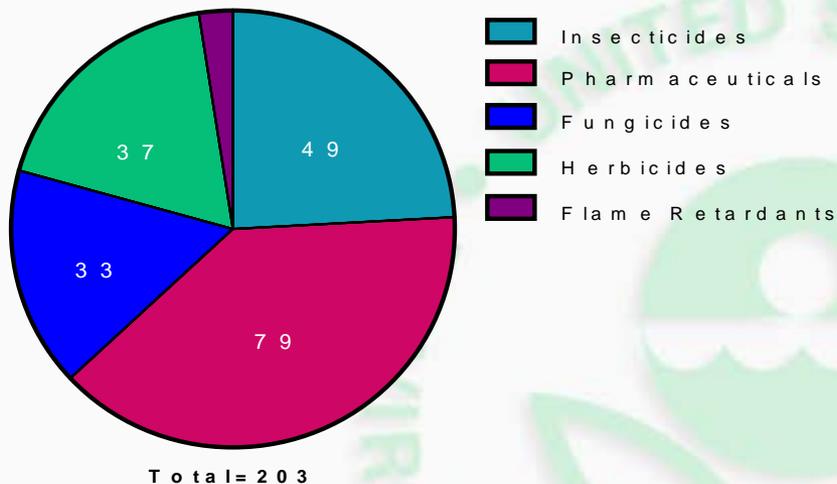
Screening ToxCast Compounds

Single concentration (40 μ M) Screen



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Five Compound Categories Accounted for 2/3 of the Hits



Categories with no hits:

Industrial
Pesticide Breakdown Product
Medicinal
Cosmetic

Compounds of Interest that Decreased MFR

<u>Organochlorines</u>	<u>Mectins</u>	<u>Pyrethroids</u>
Endosulfan	Abamectin	Allethrin
Kepone	Emamectin	Cypermethrin
Methoxychlor		Fenpropathrin
		Prallethrin
		Tetramethrin

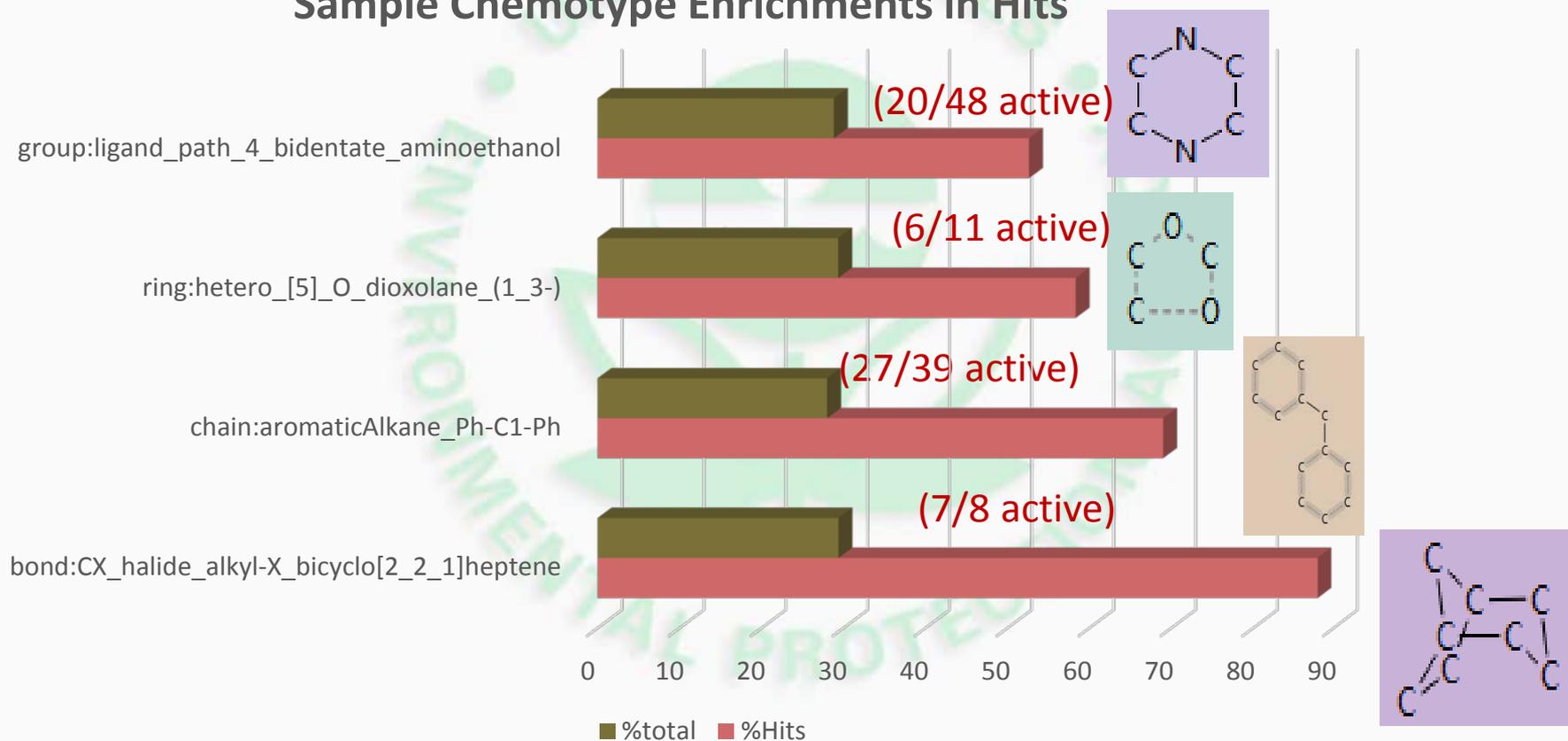
Compounds of Interest that Increased MFR

<u>Organochlorines</u>	<u>Neonicotinoids</u>
Aldrin	DDT
Endrin	DDE
Heptachlor	
Heptachlor epoxide	
Lindane	
	Nicotine
	Imidacloprid
	Thiamethoxam

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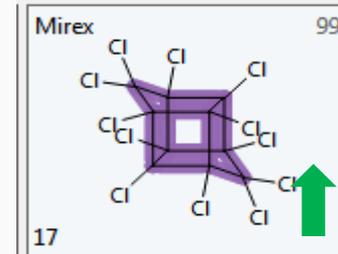
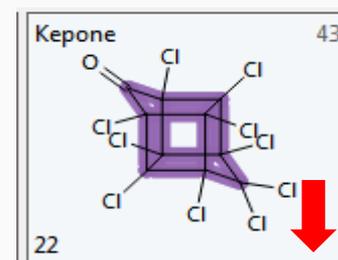
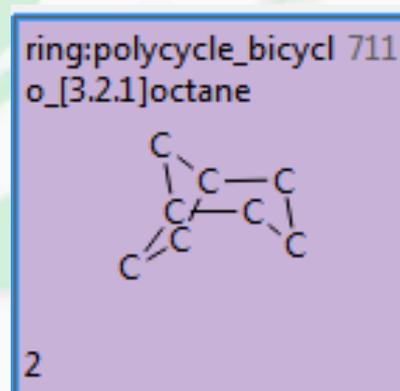
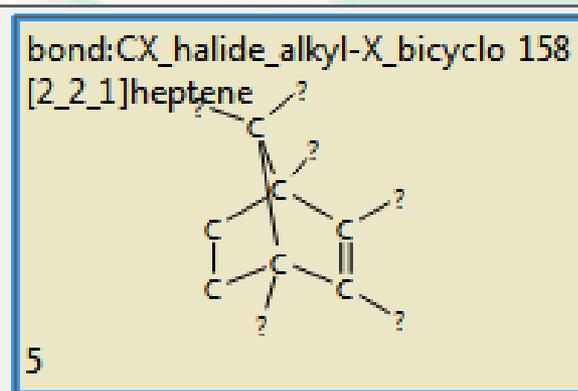
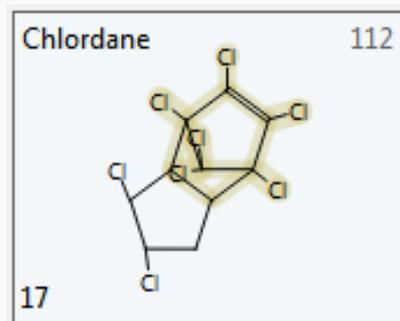
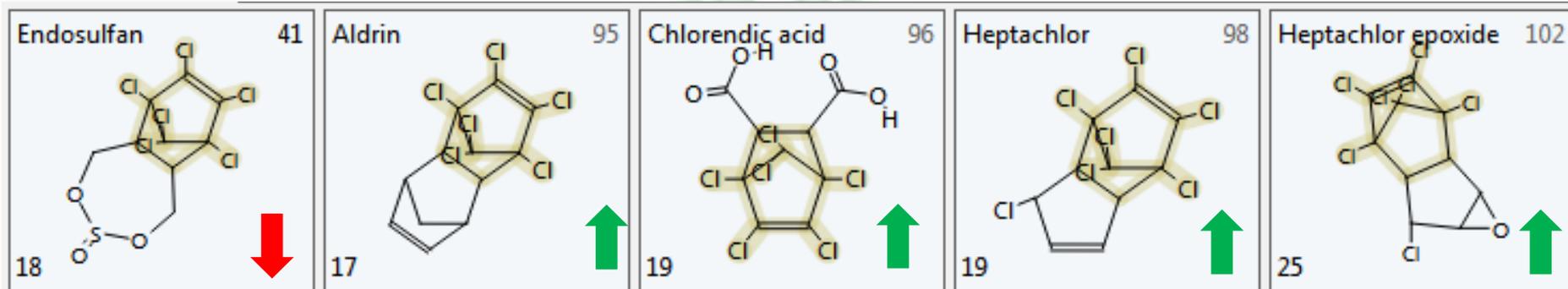
Analysis of Hits for Chemotype (CT) Enrichment

Sample Chemotype Enrichments in Hits



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7/8 of chemicals containing CTs #158 & 711 are “hits”



2/7 Hits decrease MEA activity



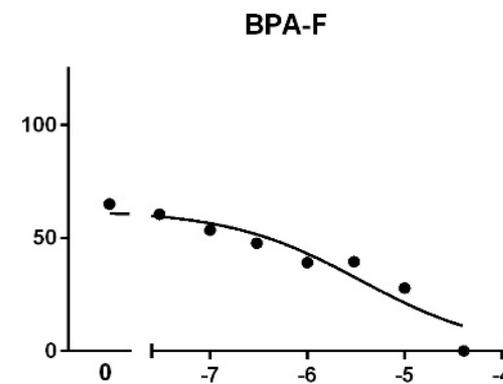
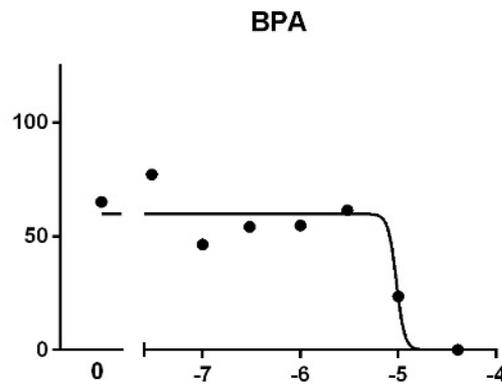
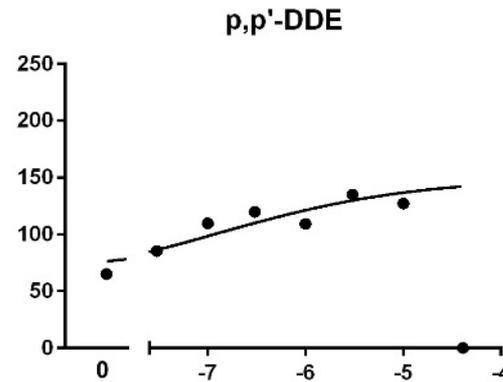
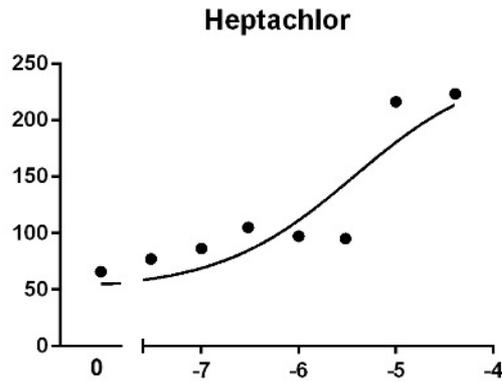
5/7 Hits increase MEA activity

This research is still in progress, and is not yet published.

Future Directions

Concentration-Response Confirmation

Mean Firing Rate (% Control)



Concentration (log M)

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HTT Task 2.2: Expanding the capability to screen and prioritize chemicals for developmental toxicity.

Faster, less expensive and predictive methods are needed for developmental neurotoxicity hazard characterization

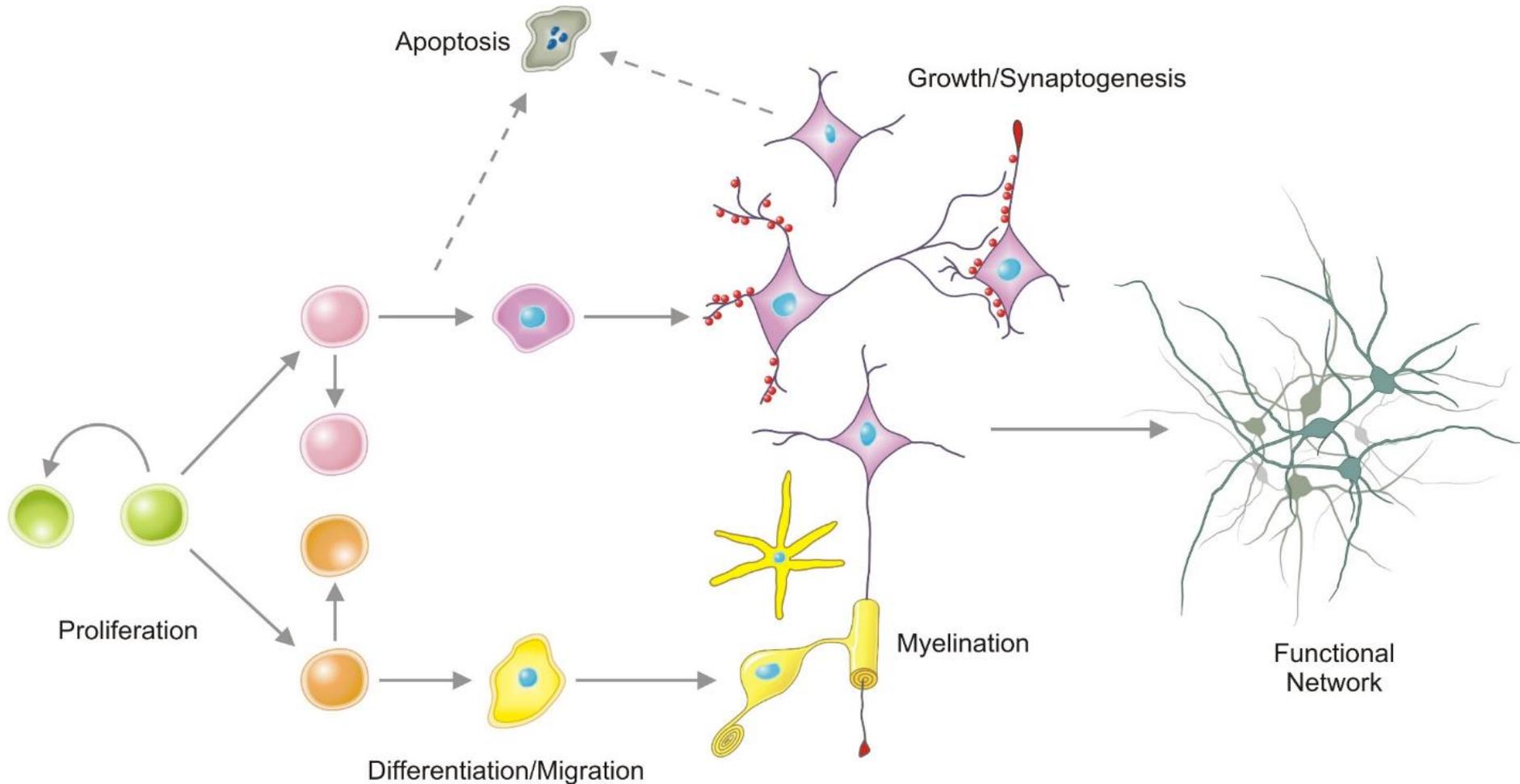
Goal

Develop medium throughput, in vitro assays to screen and prioritize chemicals for developmental neurotoxicity hazard

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Phenotypic Screening for DNT Hazard

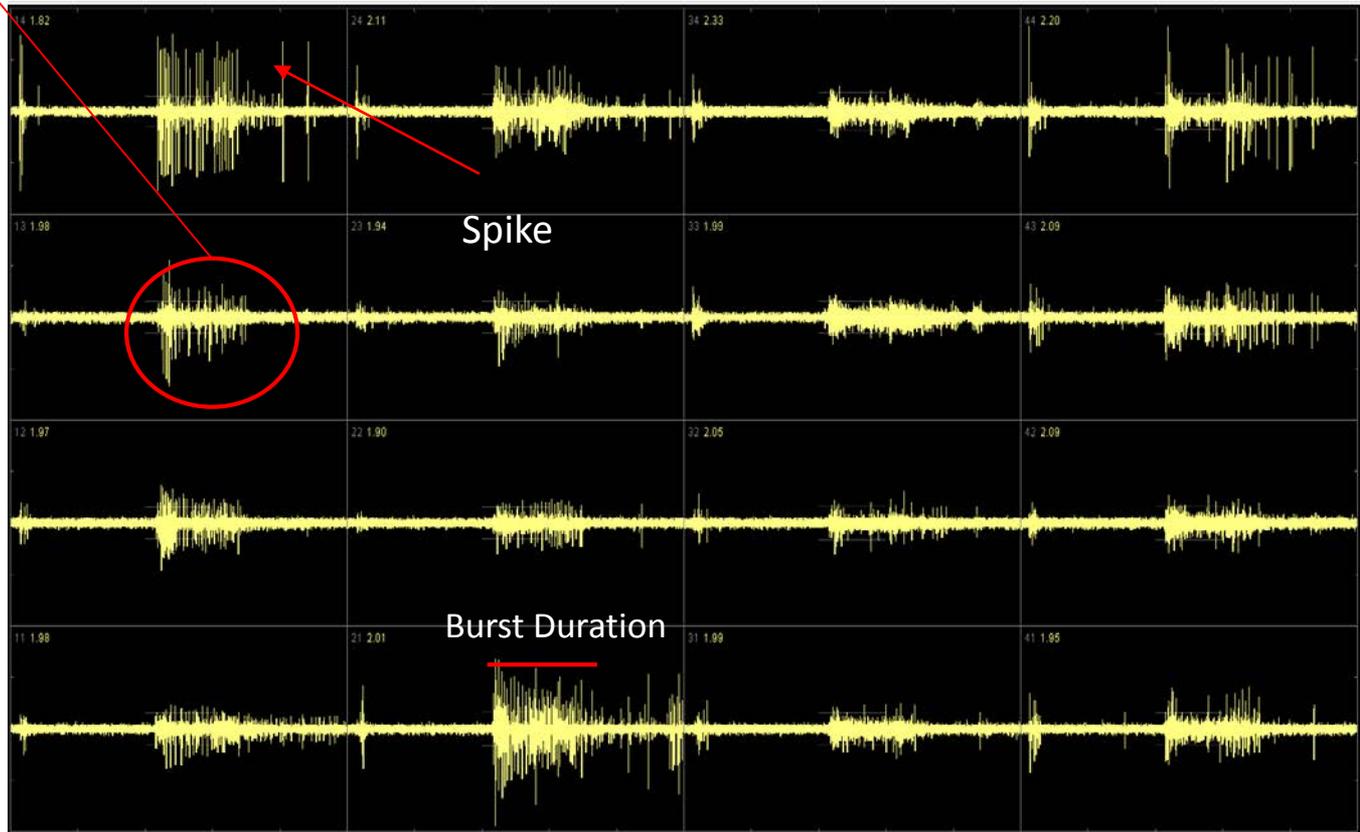
Quantify key neurodevelopmental events *in vitro*



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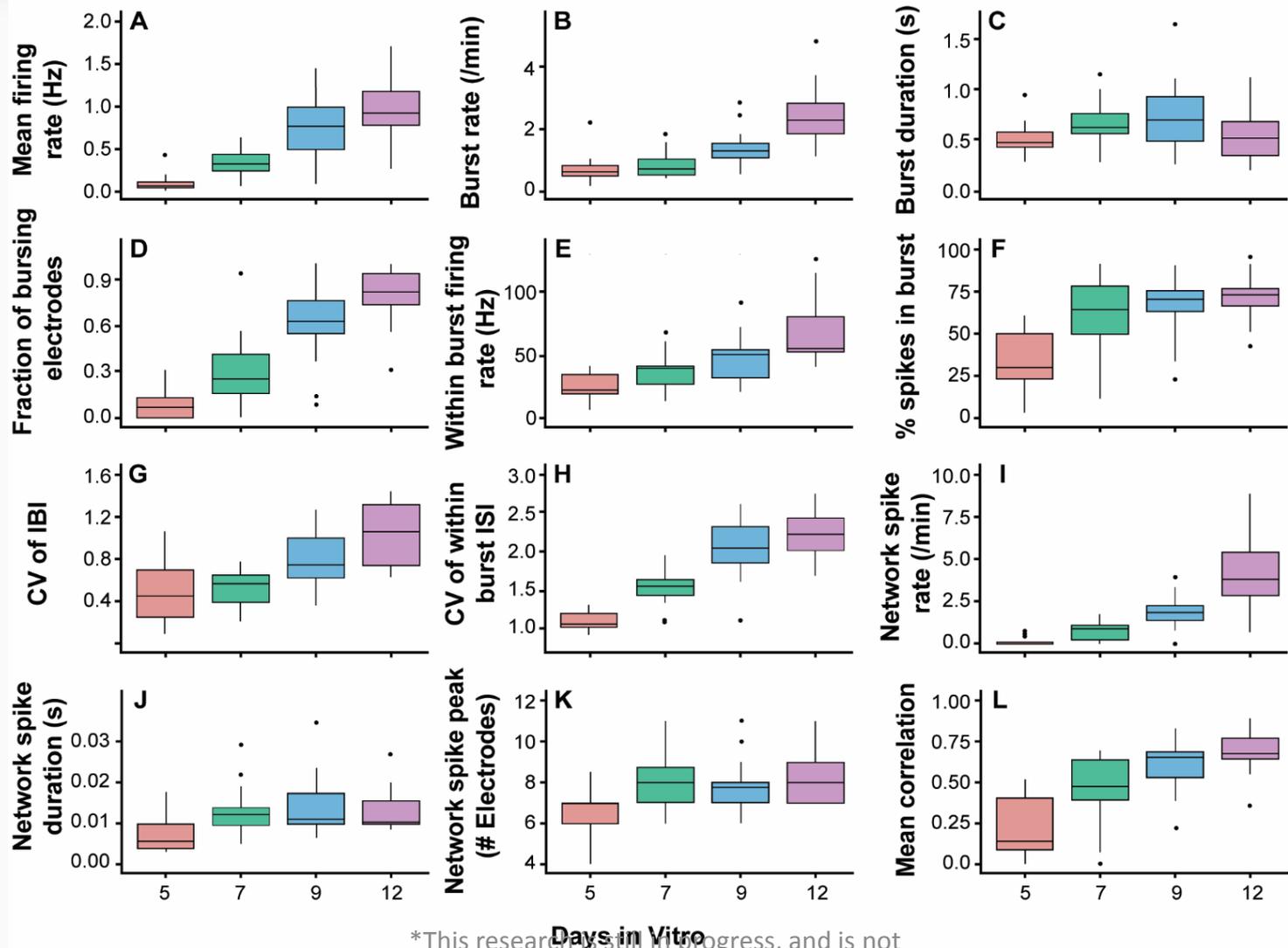
Complex network activity develops over time

Burst: A group of spikes



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Development of Network Activity on MEAs



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Developmental Neurotoxicity Screening on MEAs

Approach (PIP year 1)

Develop the Assay

- SA#1: Test Assay Positive Control Compounds

Evaluate the Assay

- SA#2: Test 30 compound DNT Reference chemicals (24 in vivo DNT, 6 negative controls)

Screen with the Assay

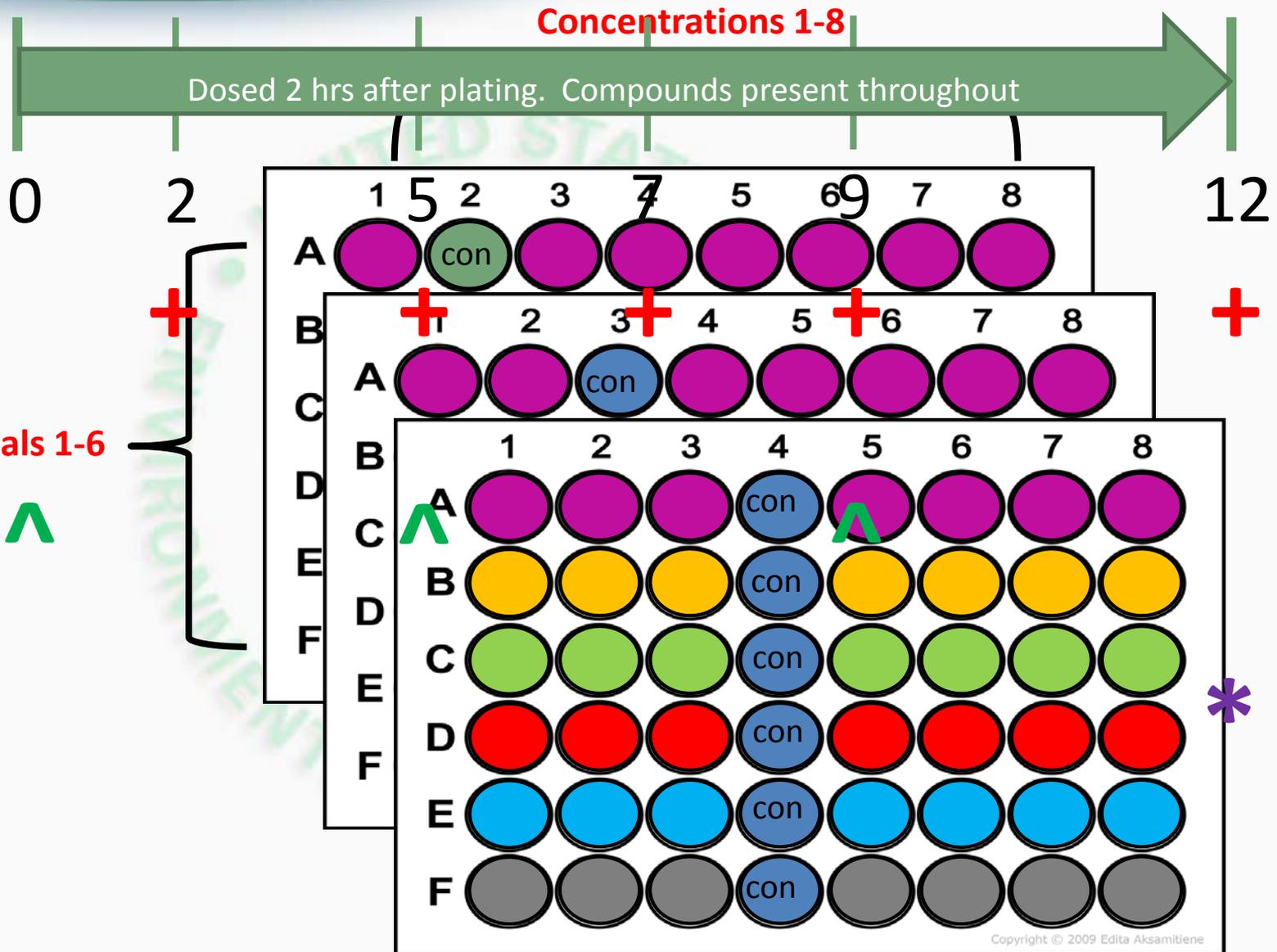
- SA#3: Screen 18 ToxCast Compounds for which acute MEA data exists

Stage 3 PIP

- SA#4: Complete screening of GRADNT compounds
 - Screen ~200 ToxCast Compounds
- SA#5 & 6. Evaluate rat and human iPS-derived neurons
- Tipping Point Analysis

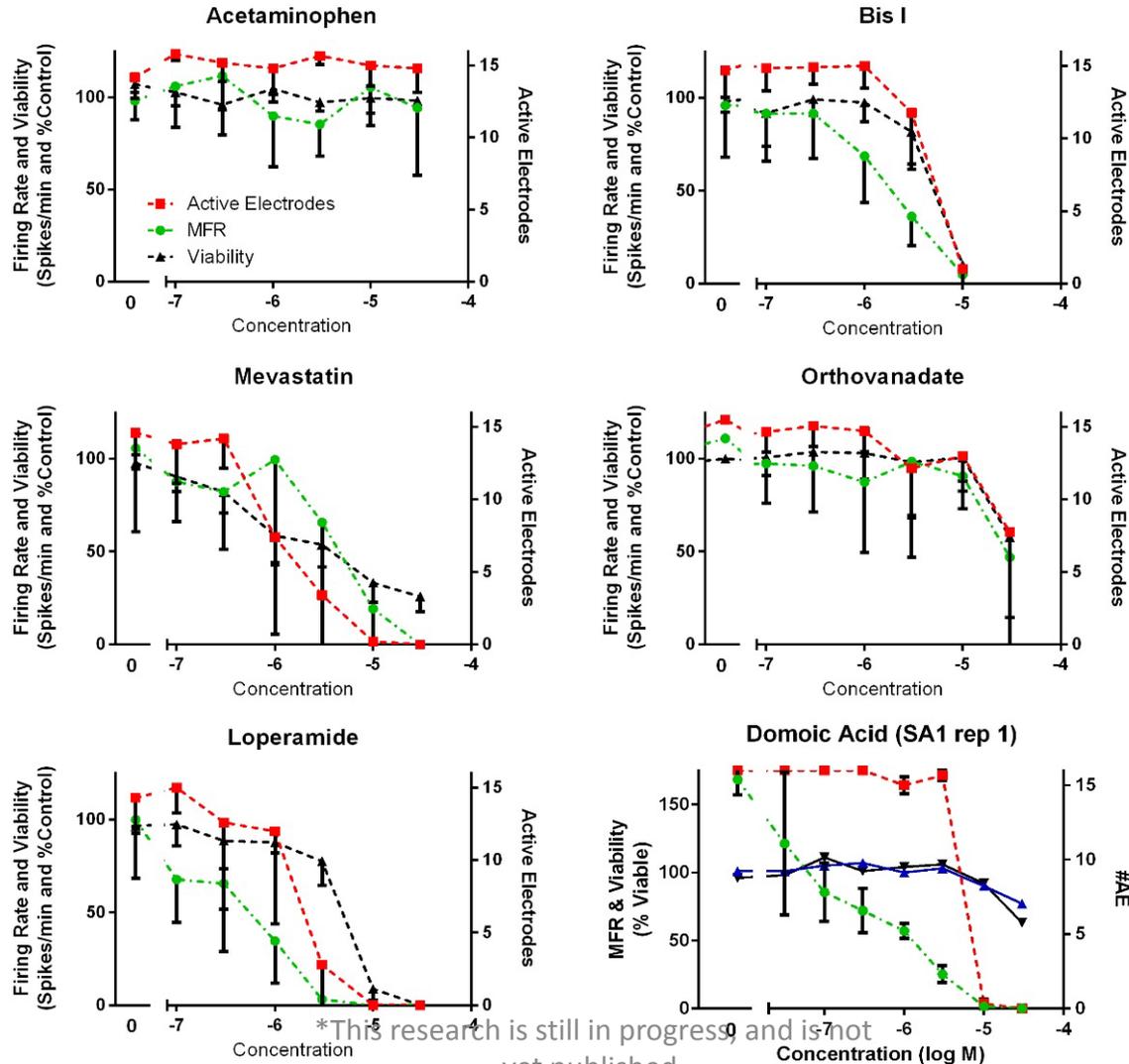
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Experimental Design



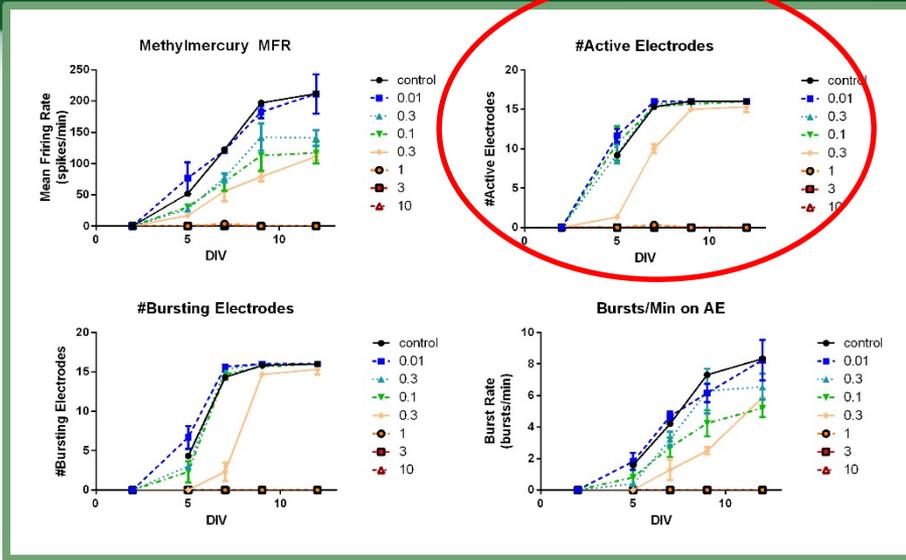
MEA/DNT Assay Development

SA#1: Test Assay Positive Control Compounds

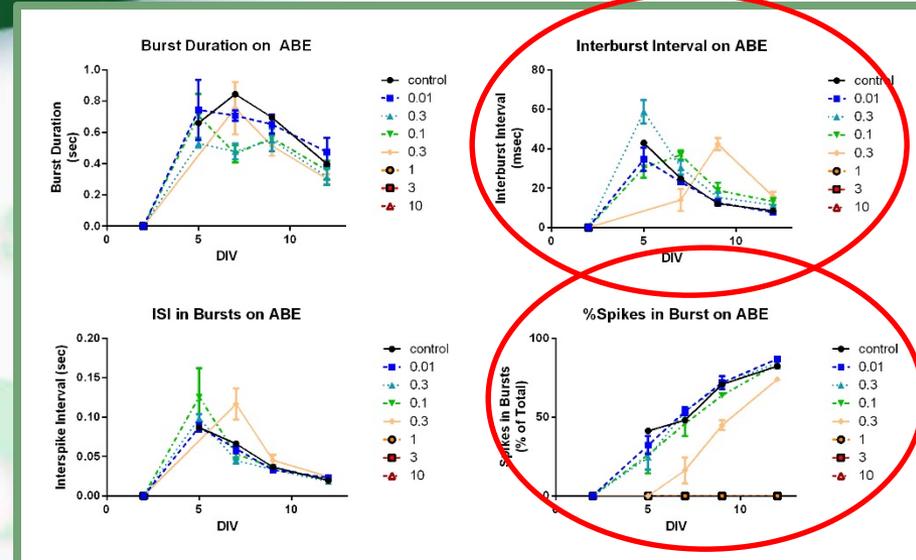


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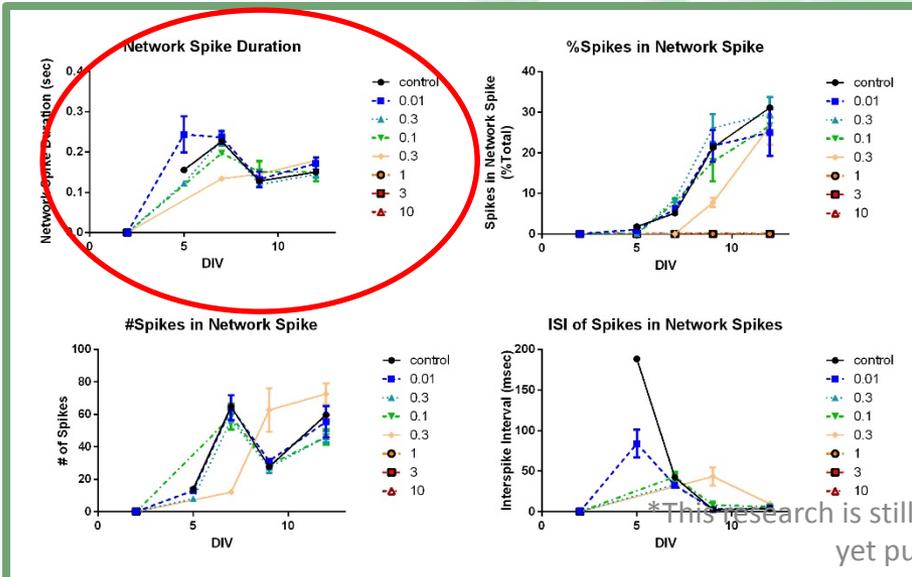
General Parameters



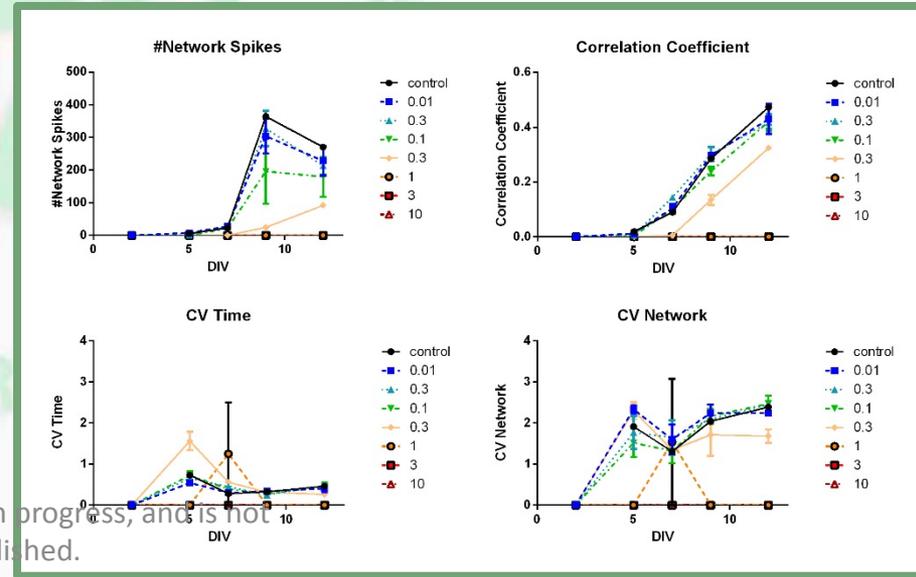
Burst Parameters



Network Connectivity Parameters



Network Spike Parameters



*This research is still in progress, and is not yet published.

Developmental Neurotoxicity Screening on MEAs

Progress to Date:

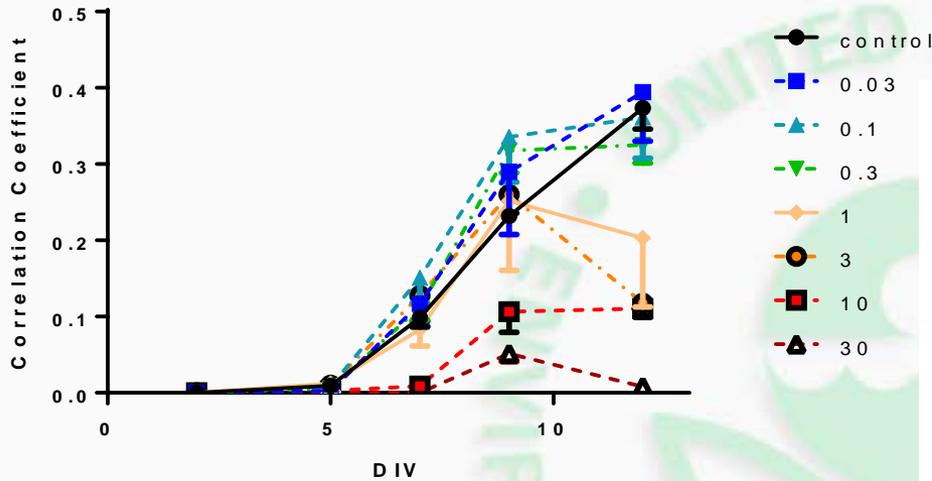
- ~100 compounds have been screened in total (excluding repeats)
 - 41 DNT reference compounds
 - 27 Organophosphate insecticides
 - 14 Negatives
 - 18 ToxCast
 - 5 Assay Controls

100 compounds X 16 endpoints X 5 timepoints X 7 concentrations =
56,000 datapoints

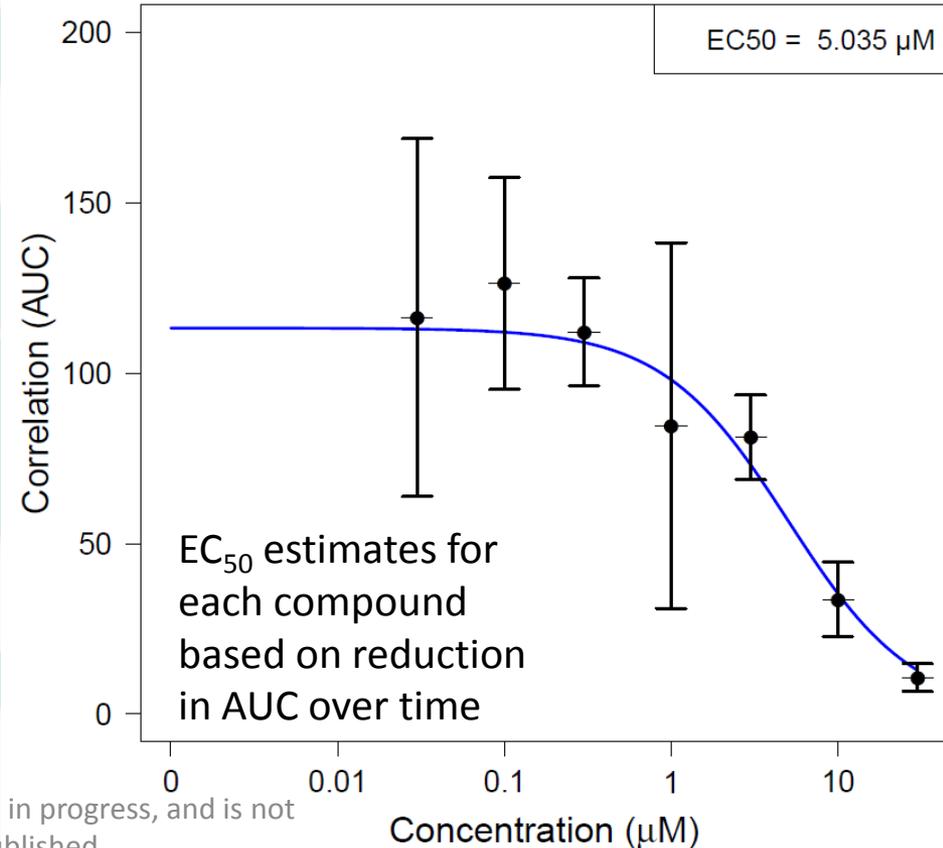
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Reducing data by collapsing across time and concentration

Correlation Coefficient

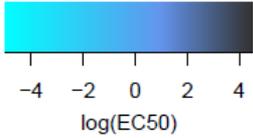


Chlorpyrifos oxon - Correlation (AUC)

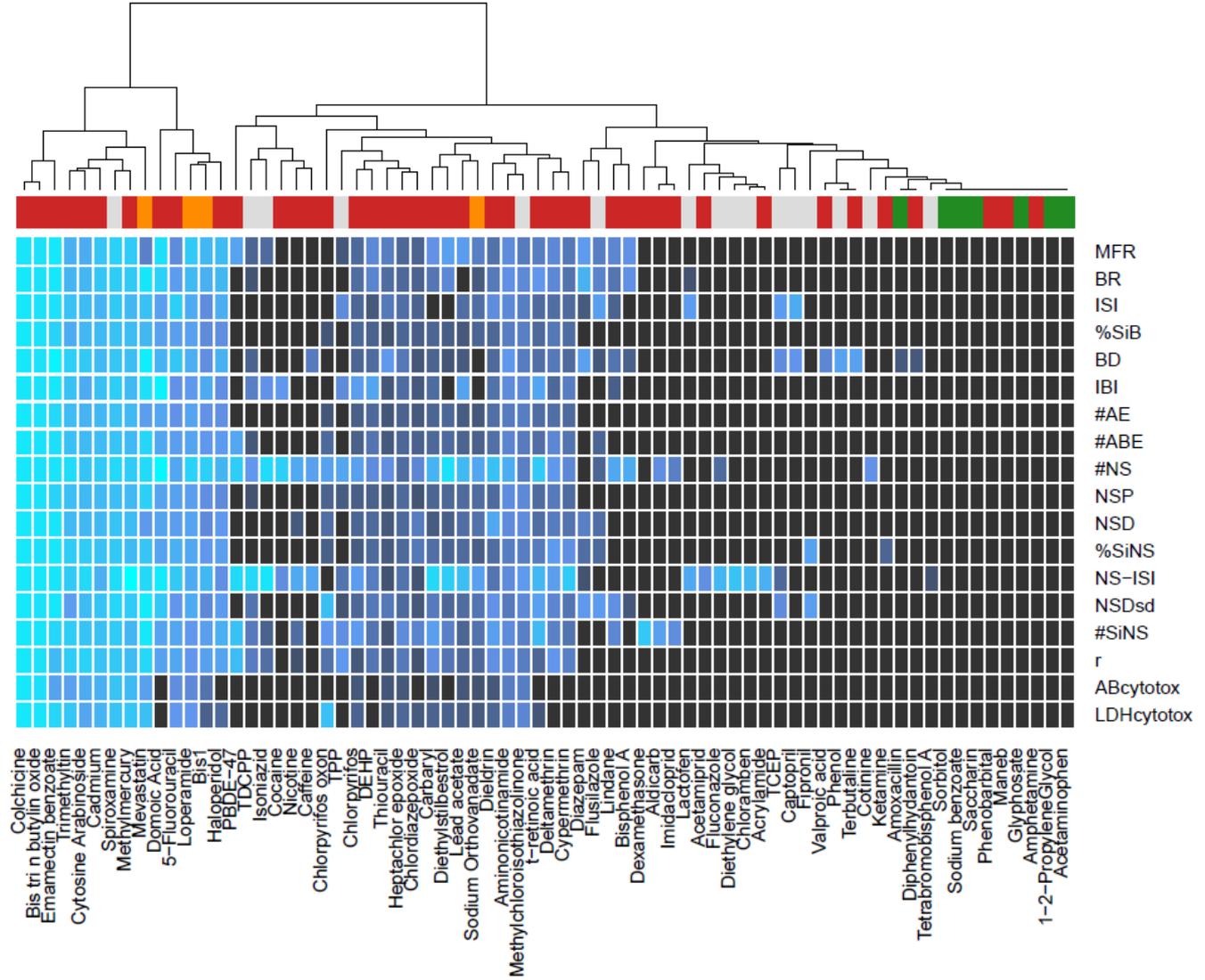


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Color Key

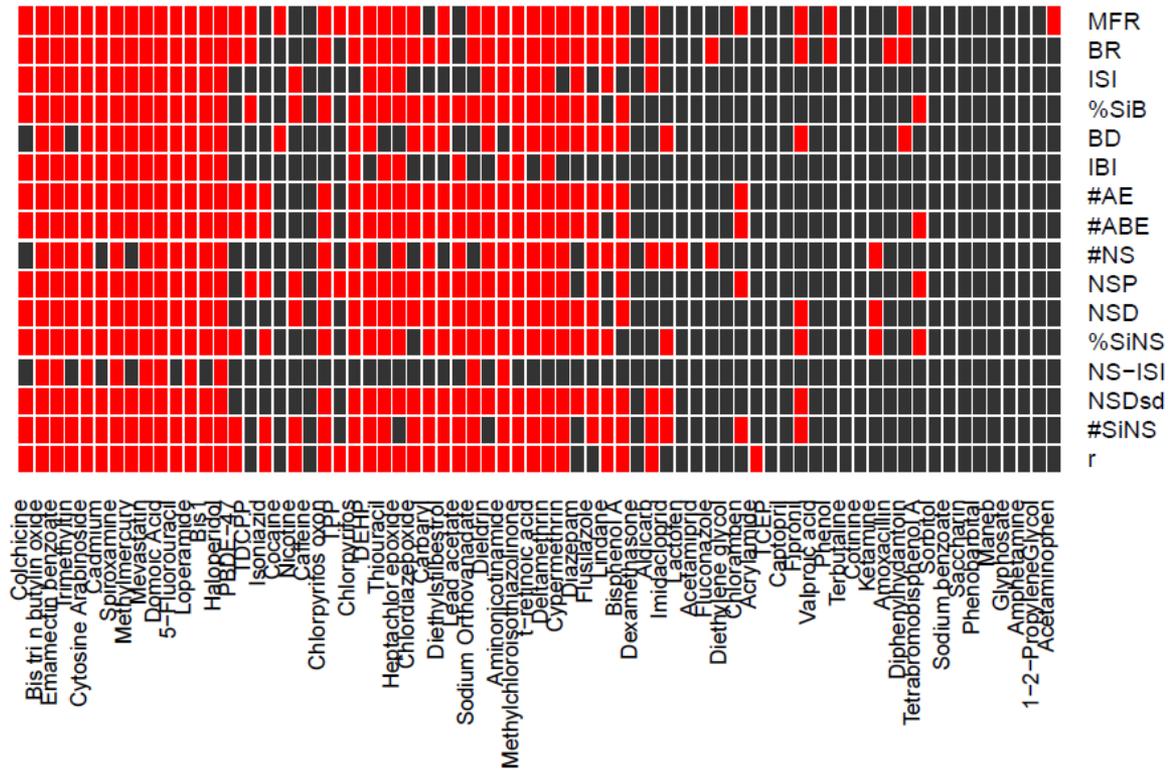
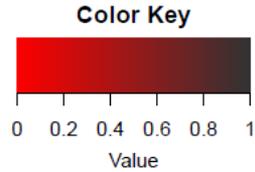


- DNT Ref
- NEG
- Unknown
- Assay +Control



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Statistically-significant Concentration-related changes



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FDR = 1%

Tipping Point:

The threshold between adaptation and adversity

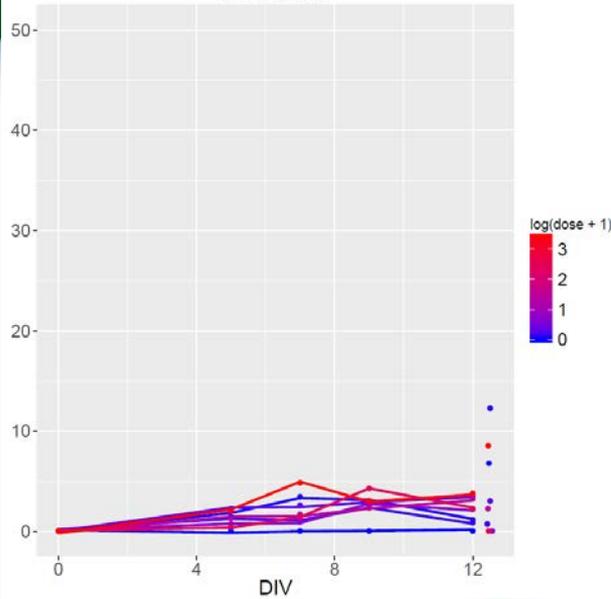
Can tipping points for chemical effects on network development be defined?

Examine MFR, BR, #NS, #AE, #ABE, correlation (r)

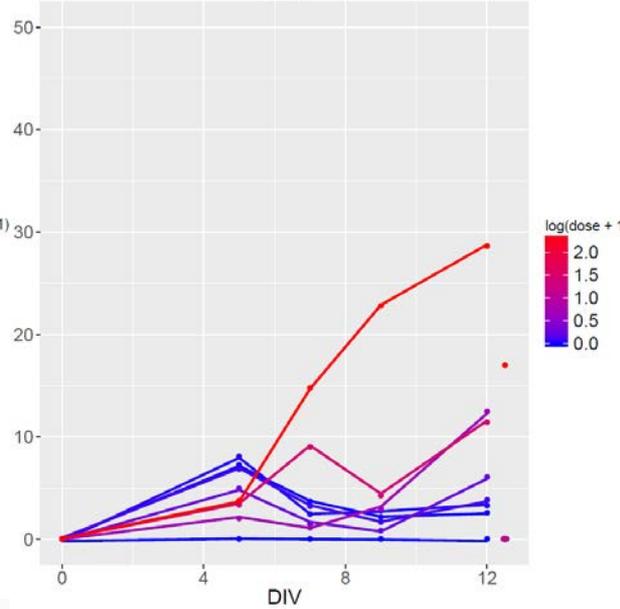
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Tipping Point Analysis- Preliminary results

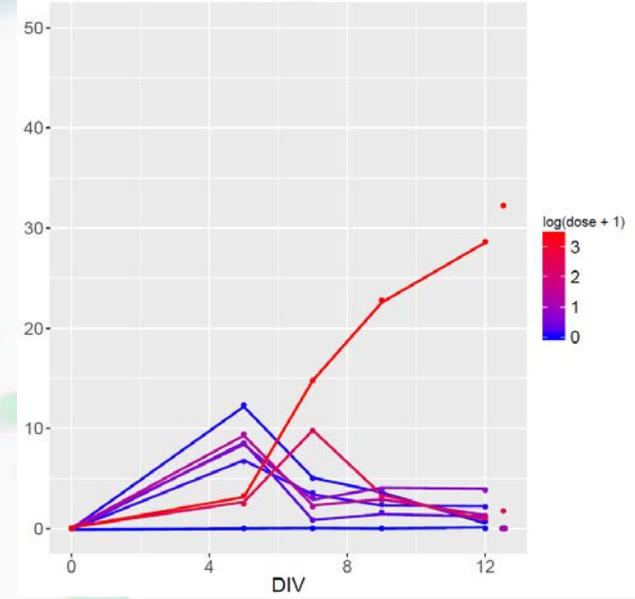
Amoxicillin



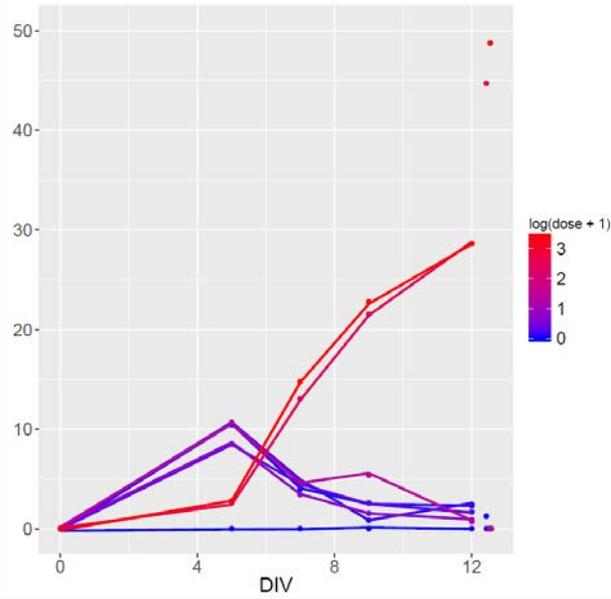
Bis1



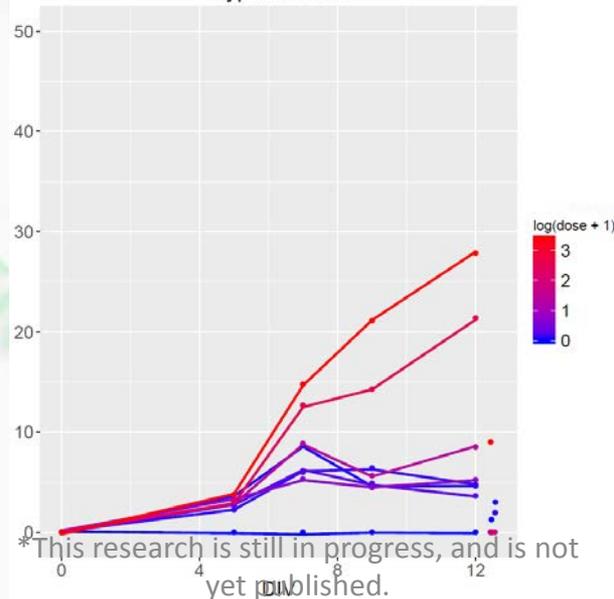
Lead acetate



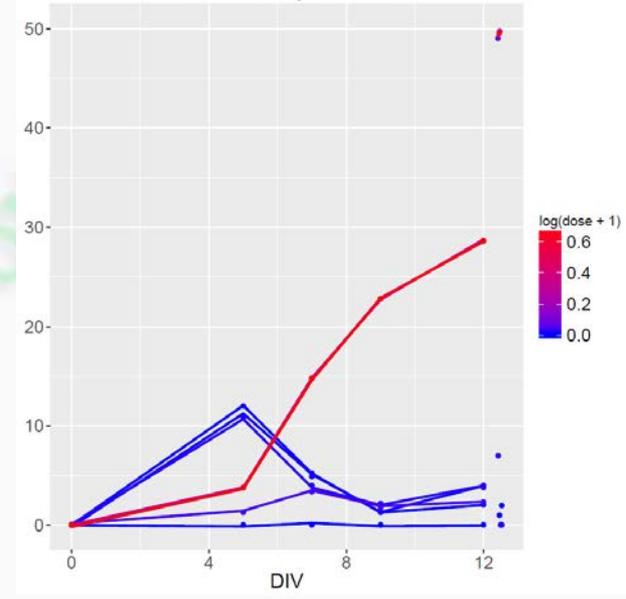
Aminonicotinamide



Cypermethrin

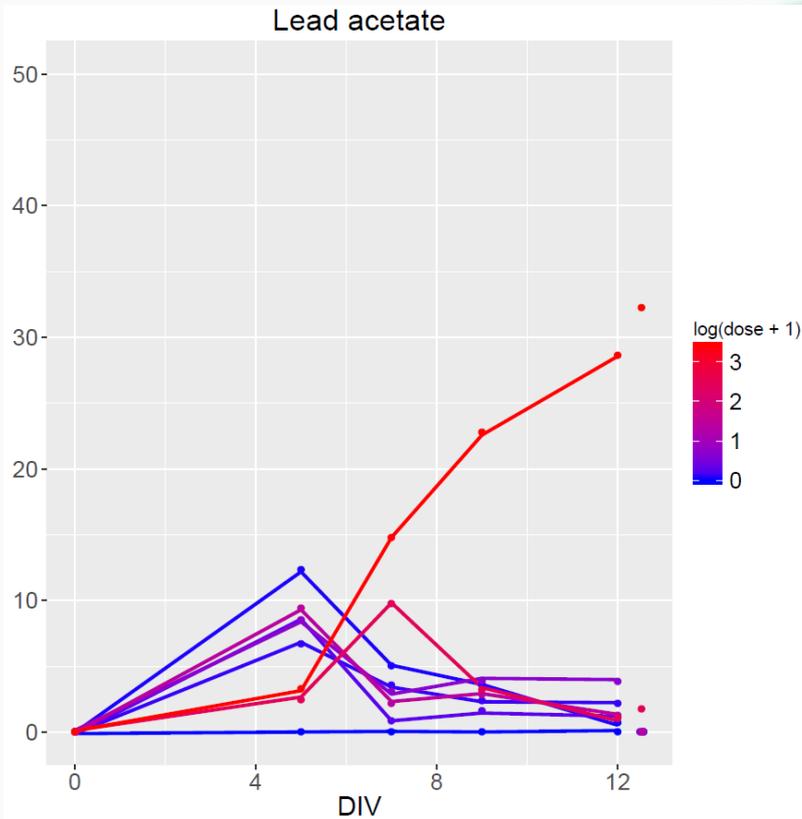


Bis tri n butylin oxide

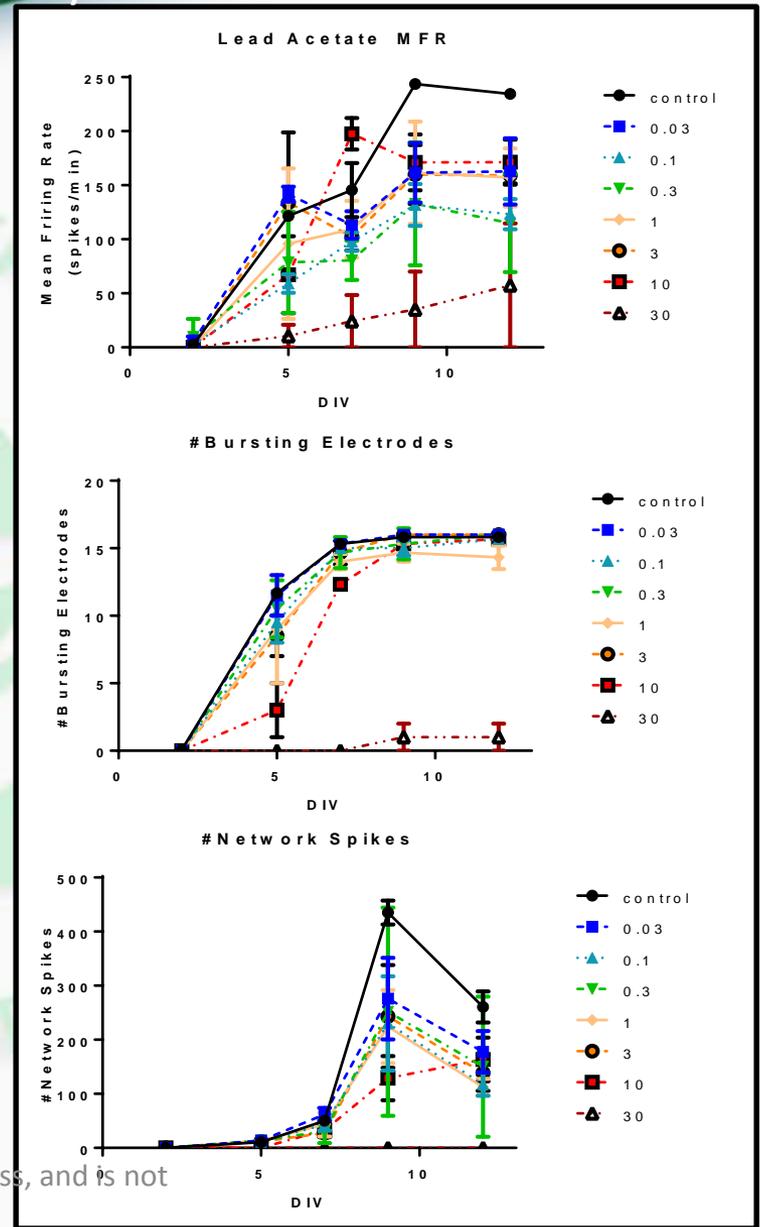


Tipping Points

In a developing network, are “adaptations” really adaptations?



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Outcomes

- Preliminary Data Analysis indicates that MEA assay can separate developmentally neurotoxic from non-neurotoxic compounds.
 - Analysis is ongoing
- Screening compounds at a rate of ~100/year; ~\$300/cmpd
 - Compare to ~2/yr & ~\$900,000/cmpd for Guideline DNT assay
 - <100 environmental compounds screened in 20 years in Guideline DNT

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Future Directions

- Continue Screening
 - Finish the ~100 DNT reference compounds
 - Screen ToxCast Acute MEA Hits
 - Goal is ~300 compounds in 2 years
 - Includes High Priority Compounds (e.g. Flame Retardants, OP insecticides)
- Continue Tipping Point Analysis
- Develop “Animal Free” Complex Cell Models
 - Rodent Stem Cell Models
 - Human Stem Cell Models
 - Identify viable models and begin screening DNT “reference” compounds

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Acknowledgements:

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- Jasmine Brown

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