Evaluating neurotoxicity hazard using human and rodent neural networks

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Outline

I. Brief History of the EPA and Regulatory Statutes
II. The Need for Alternative Approaches for Neurotoxicity and Developmental Neurotoxicity Hazard Assessment
III. Introduction to MicroElectrode Array (MEA) recording
    I. Rodent Primary Cortical Neurons
    II. NeuCyte human IPS-derived neurons
IV. Assessing Acute Neurotoxicity Hazard with MEAs
V. Assessing DNT Hazard with MEAs
VI. Informing AOP Development with MEA Data
VII. Future Directions
VIII. Questions

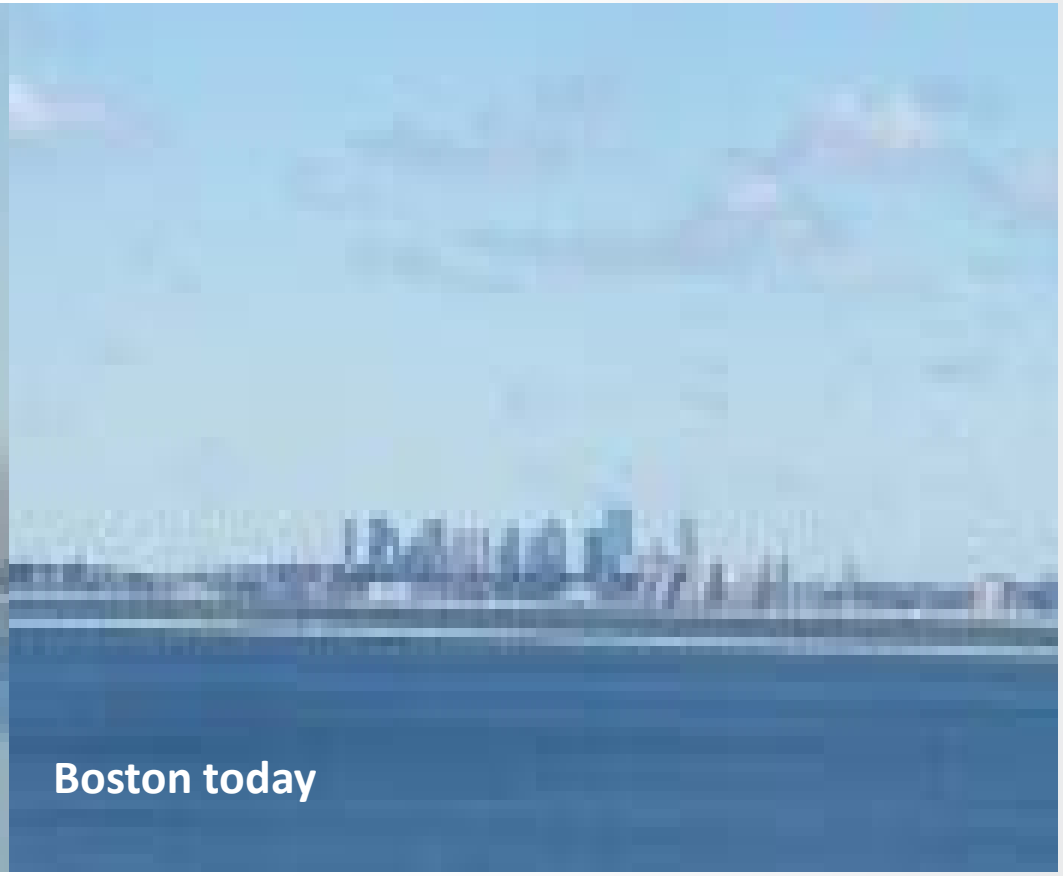
History of the EPA

- Republican President Richard Nixon established the EPA in 1973
  - Unambiguous pollution issues
  - Pb in gasoline; smog; water pollution; failure of raptor nesting

Smokey Skies in Birmingham, 1972, epa.gov

Los Angeles Smog, 1972, epa.gov

EPA has made a difference...

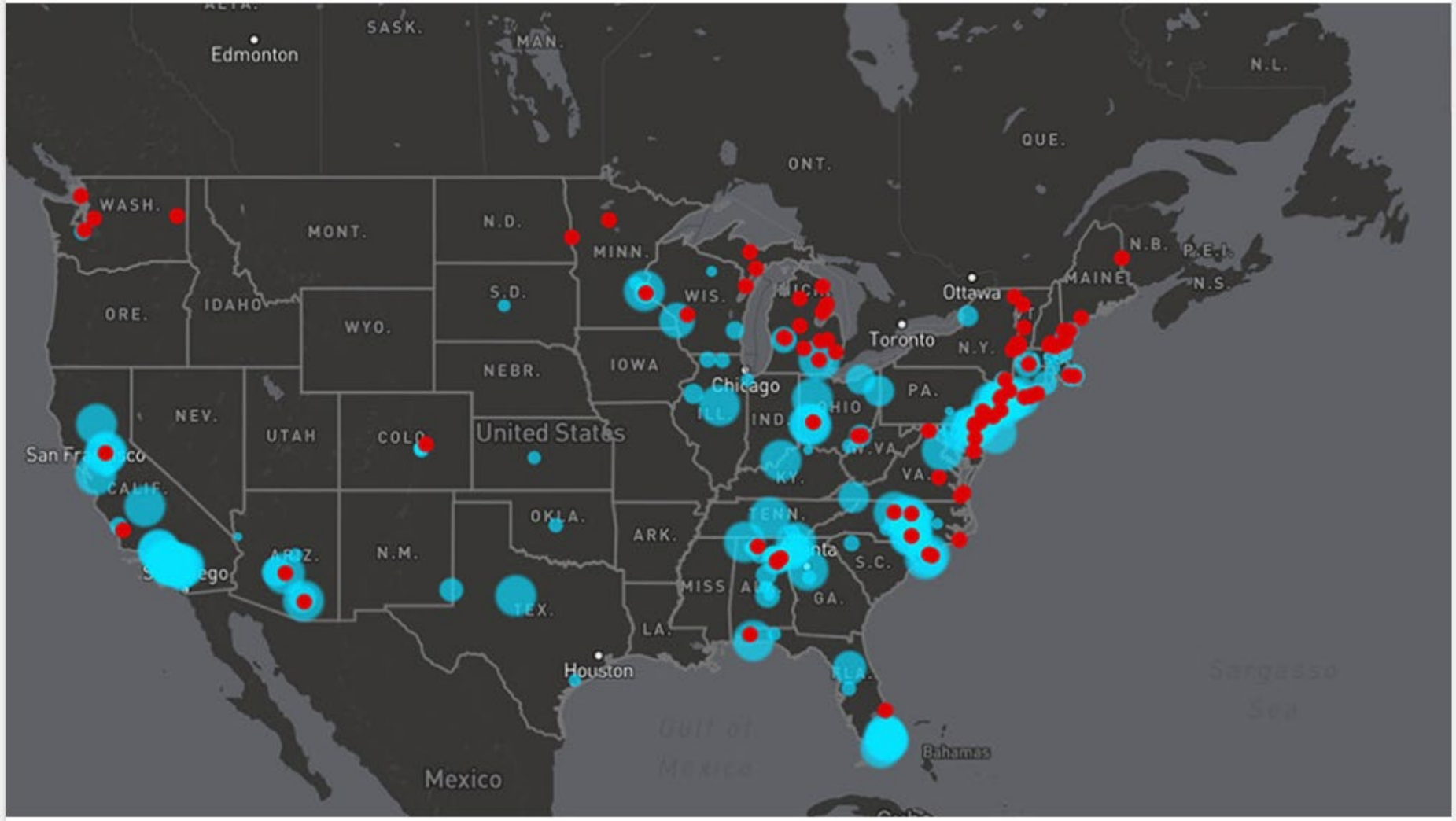


https://www.epa.gov/history/historical-photos-and-images

The EPA is still needed

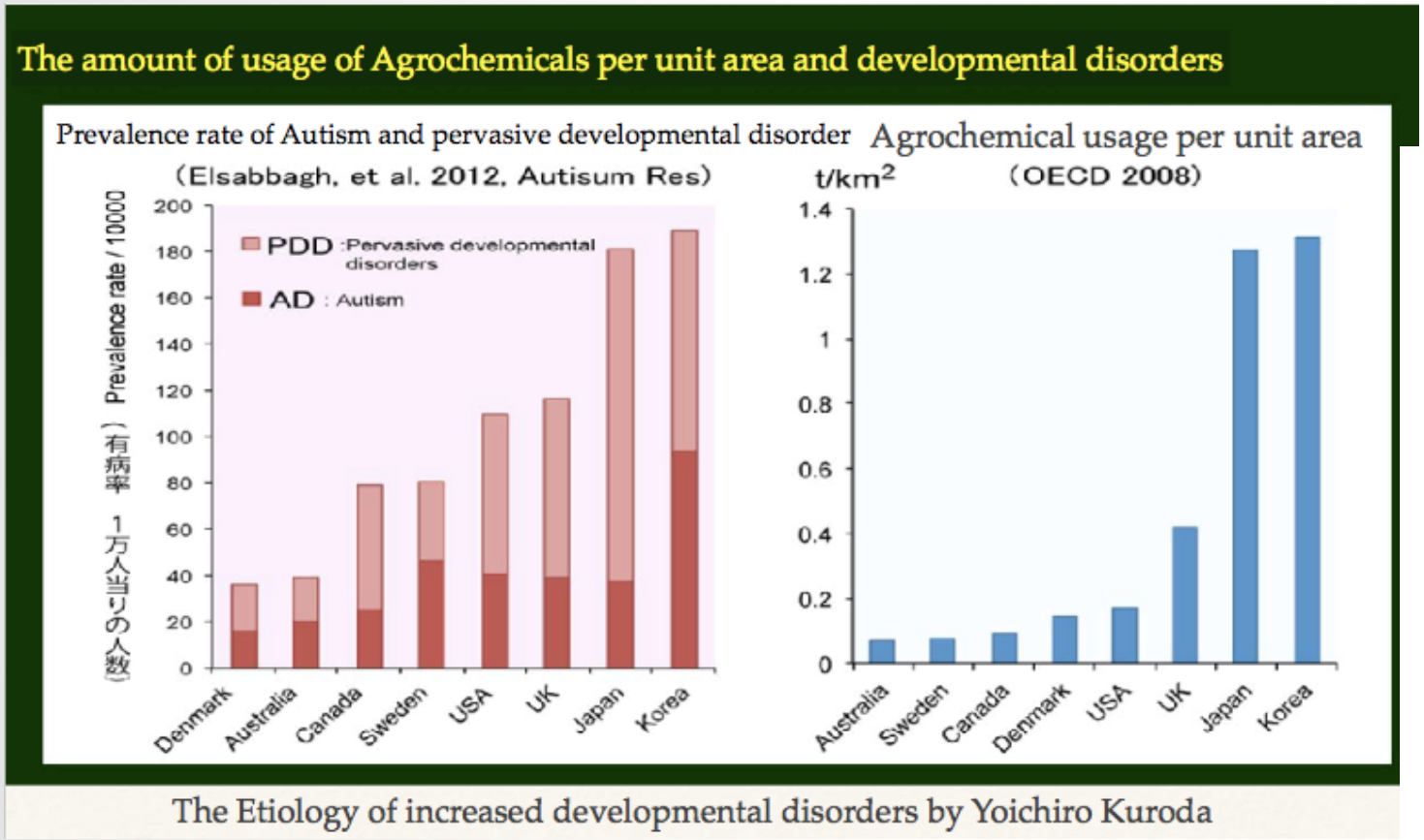
PFAS contamination continue to surface at Van Etten Lake (Oscoda County, MI)

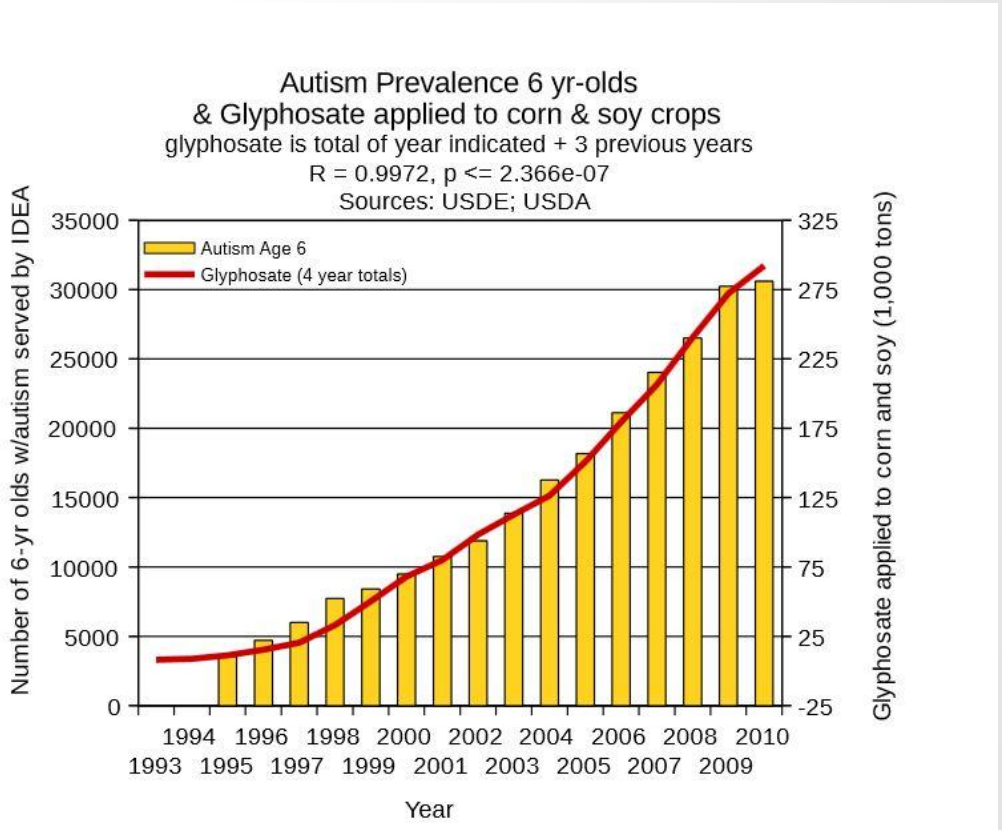
PFAS are a national problem



Toxic fluorinated chemicals in tap water and at industrial or military sites. *Environmental Working Group*

Developmental neurotoxicity is a public concern





The mission of EPA is to protect human health and the environment

What does EPA do to accomplish this mission?:

- Sets standards (limits) for chemicals in the environment.
- Registers chemicals (develop guidelines).
- Develops pollution prevention technology.
- Conducts Risk Assessments (based on sound science).
- Informs and educates the public.
- **Conducts Research to provide a solid scientific basis for all of the above activities**.

EPA's Research is Centered Around Regulatory Needs

Legislation	Acronym	Primary EPA Program Office	ORD Research Program
Clean Air Act	CAA	OAR	Air and Energy (A-E)
Clean Water Act	CWA	OW	Safe and Sustainable Water Resources (SSWR)
Comprehensive Environmental Response, Compensation, and Liability Act	CERCLA	OLEM	Safe and Healthy Communities (SHC) & Homeland Security (HS)
Federal Food, Drug, and Cosmetic Act	FFDCA	OCSPP/OPP	
Federal Insecticide, Fungicide, and Rodenticide Act	FIFRA	OCSPP/OPP	Chemical Safety for Sustainability (CSS)
Food Quality Protection Act	FQPA	OCSPP/OPP/OW	CSS
National Environmental Policy Act	NEPA		
Resource Conservation and Recovery Act	RCRA	OLEM	SHC
Safe Drinking Water Act	SDWA	OW	SSWR & HS
Toxic Substances Control Act	**TSCA**	**OCSPP/OPPT**	CSS

The Differences between TSCA and FIFRA

Toxic Substances Control Act (TSCA)

All New Chemicals
>60-80K "Grandfathered" Chemicals ("existing" chemicals)

Available Data
90 Day Premanufacture Notice

"Data Poor"- little or nothing may be known about toxicity hazard

Intended to Kill Something

Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)

All "Pesticides"

Required Guideline Studies
Health and Environmental Effects

Data Rich- Toxicity hazard is well characterized

Lautenberg Chemical Safety Act 2016

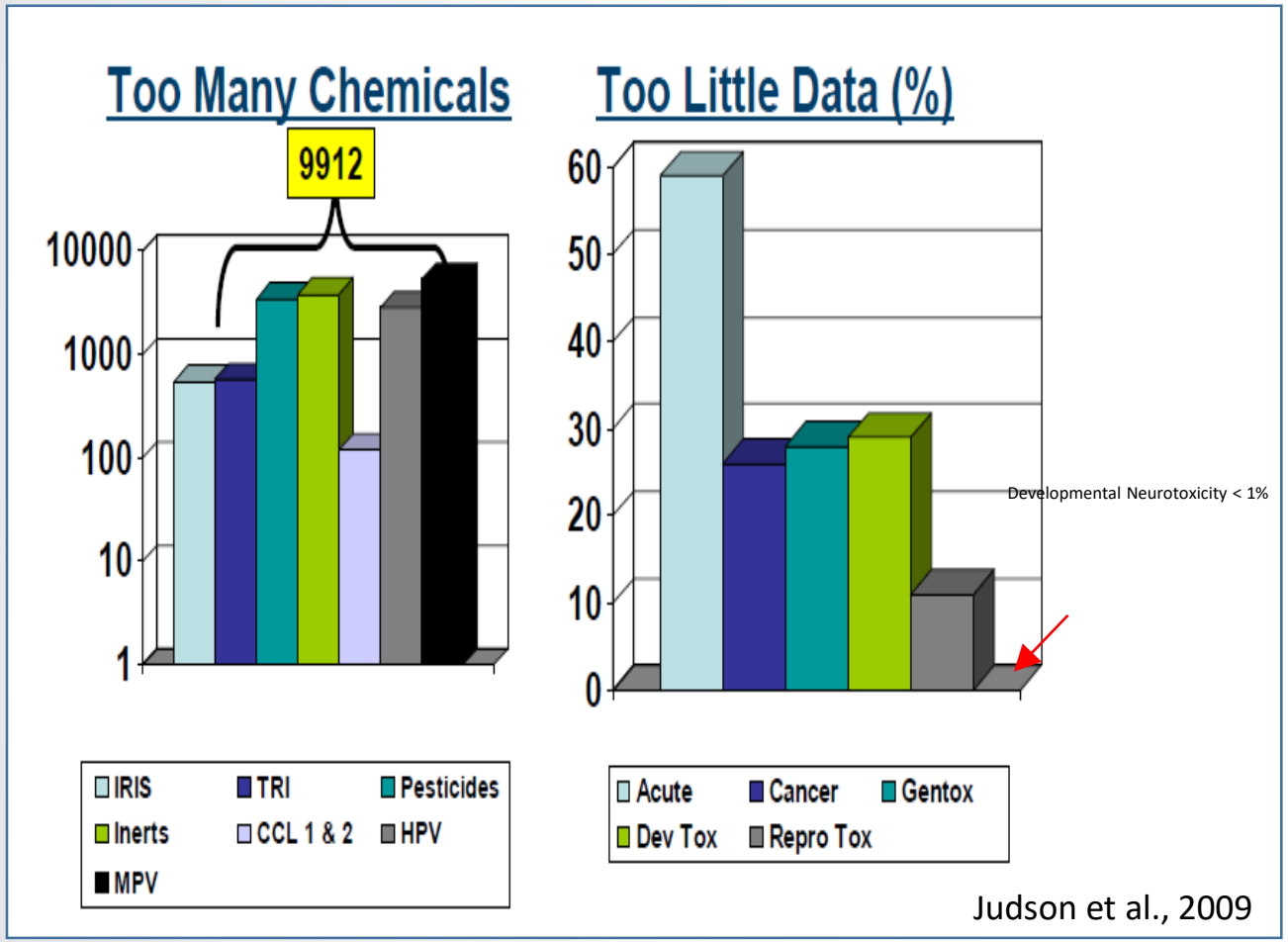
- **Mandatory requirement for EPA to evaluate existing chemicals with clear and enforceable deadlines;**
- **Risk-based chemical assessments;**
- **Increased public transparency for chemical information;**
- **Consistent source of funding for EPA to carry out the responsibilities under the new law.**
- **Must consider risks to susceptible and highly exposed populations**
- **Directs EPA to utilize alternatives to animals**

Food Quality Protection Act of 1996

- **Mandates an extra 10x safety factor for children/infants**
- **Mandates Assessment of Cumulative Risk to Pesticides with the same mode of action**

What is driving the push to New Alternative Methods (NAMs) for Neurotoxicity and Developmental Neurotoxicity?

Many Chemicals Lack Developmental Neurotoxicity (DNT) Data



Judson et al., 2009

Current testing too slow

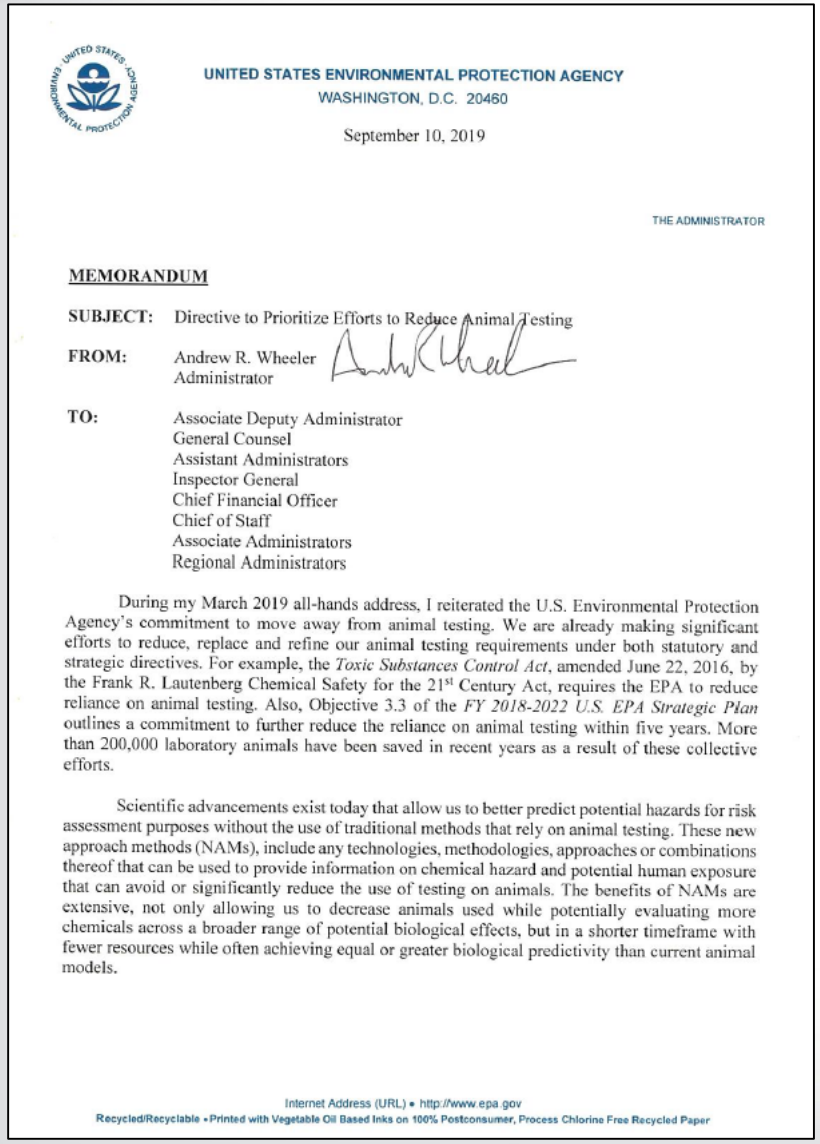
- Not Required under FIFRA
- Animal "Guideline" DNT; 1 chemical, $1M cost; 2 yr
- At current pace, ~150 chemicals in 20+ yrs
- Not often used (~25%) for point of departure values for risk assessment*

The absence of DNT hazard data on chemicals impedes consideration of this adverse outcome in environmental decision-making.

Reports of the potential involvement of environmental chemicals in increased rates of neurodevelopmental disease contributed to increasing public concern about DNT hazard of chemicals

*Raffaele et al. The use of **developmental neurotoxicity** data in pesticide risk assessments. Neurotoxicol Teratol. 2010 Sep-Oct;32(5):563-72.

EPA-Specific Drivers



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

September 10, 2019

THE ADMINISTRATOR

MEMORANDUM

SUBJECT: Directive to Prioritize Efforts to Reduce Animal Testing

FROM: Andrew R. Wheeler
Administrator

TO: Associate Deputy Administrator
General Counsel
Assistant Administrators
Inspector General
Chief Financial Officer
Chief of Staff
Associate Administrators
Regional Administrators

During my March 2019 all-hands address, I reiterated the U.S. Environmental Protection Agency's commitment to move away from animal testing. We are already making significant efforts to reduce, replace and refine our animal testing requirements under both statutory and strategic directives. For example, the *Toxic Substances Control Act*, amended June 22, 2016, by the Frank R. Lautenberg Chemical Safety for the 21st Century Act, requires the EPA to reduce reliance on animal testing. Also, Objective 3.3 of the *FY 2018-2022 U.S. EPA Strategic Plan* outlines a commitment to further reduce the reliance on animal testing within five years. More than 200,000 laboratory animals have been saved in recent years as a result of these collective efforts.

Scientific advancements exist today that allow us to better predict potential hazards for risk assessment purposes without the use of traditional methods that rely on animal testing. These new approach methods (NAMs), include any technologies, methodologies, approaches or combinations thereof that can be used to provide information on chemical hazard and potential human exposure that can avoid or significantly reduce the use of testing on animals. The benefits of NAMs are extensive, not only allowing us to decrease animals used while potentially evaluating more chemicals across a broader range of potential biological effects, but in a shorter timeframe with fewer resources while often achieving equal or greater biological predictivity than current animal models.

Internet Address (URL) • http://www.epa.gov
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USEPA Administrator Memo Prioritizing Efforts to Reduce Animal Testing, September 10, 2019

- EPA will reduce its requests for, and our funding of, mammal studies by 30 percent by 2025
- EPA will eliminate all mammal study requests and funding by 2035.
- Form a working group of agency experts in this field who will provide a work plan within six months.
- https://www.epa.gov/environmental-topics/administrator-memo-prioritizing-efforts-reduce-animal-testing-september-10-2019

The Differences between TSCA and FIFRA

TSCA		FIFRA
All New Chemicals >60-80K “Grandfathered” Chemicals	Intended to Kill Something →	All “Pesticides”
Available Data 90 Day Premanufacture Notice		Required Guideline Studies Health and Environmental Effects
“Data Poor”- little or nothing may be known about toxicity hazard		Data Rich- Toxicity hazard is well characterized
Need: Data of any kind on NT and/or DNT.		Need: Data that can support “fit for purpose” decision-making.

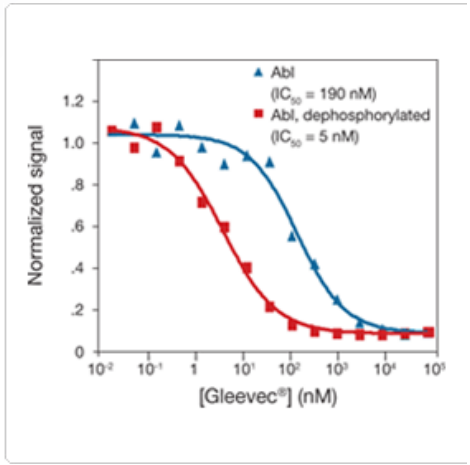
How do we address these challenges for neurotoxicity and DNT?

Solution: Faster, inexpensive and predictive methods are needed to detect and characterize compounds with developmental neurotoxicity hazard

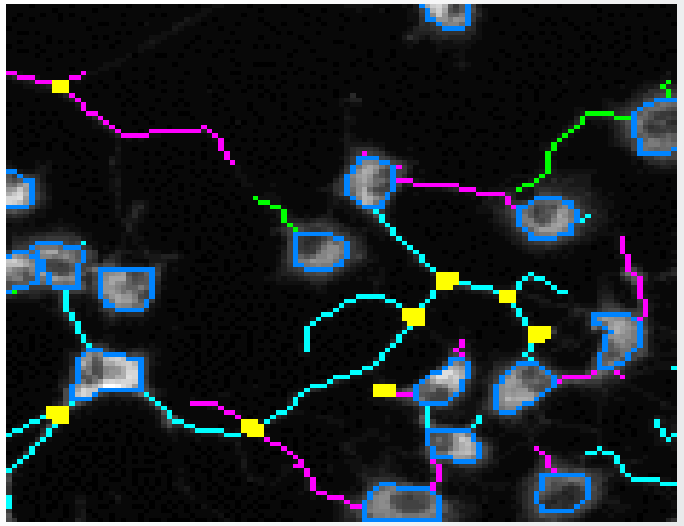
- Develop high throughput, in vitro assays,
- Characterize chemicals for neurotoxicity and developmental neurotoxicity hazard
- Data from these assays can provide information for decision-making

Approaches for Neurotoxicity NAMs

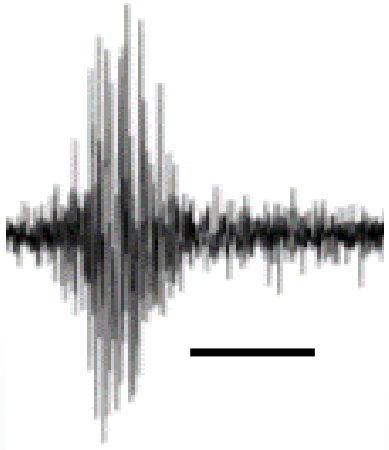
Biochemical Endpoints
(e.g. ToxCast)
•ion channels
•AChE
•thyroid hormone metabolism
•growth factor receptors
•cell adhesion molecules



Morphological Endpoints
•Neurite outgrowth
•Cell type
•Synapse number
•Proliferation



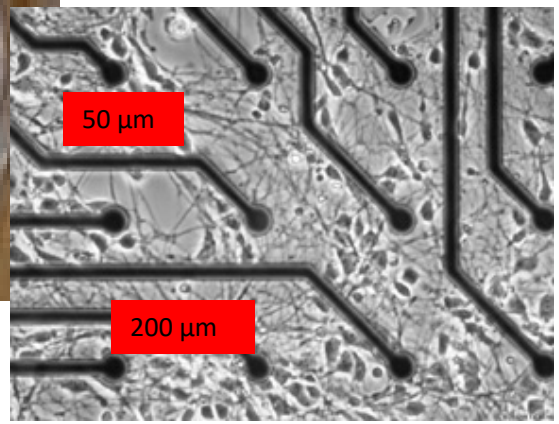
Functional Endpoints
•Patch clamp electrophysiology
•Ion homeostasis (e.g. Calcium imaging)
•Membrane potential
•Mitochondrial Function
•Microelectrode array (MEA) recording



Network Function is Crucial to the Nervous System

- Spiking, bursting, and synchronous activity are intrinsic network functions.
  - These properties of networks develop spontaneously *in vivo* and *in vitro*
- Neuro-developmental processes are influenced by electrical activity.
- Patterns of network activity are highly conserved.
  - There is greater similarity across the same region of brain from different species than between brain regions of the same species
- Synchronous activity in networks is integral to sensory awareness, attention, memory and other cognitive processes.

Measurement of Network Function and Formation in vitro using Microelectrode Array (MEA) Recording

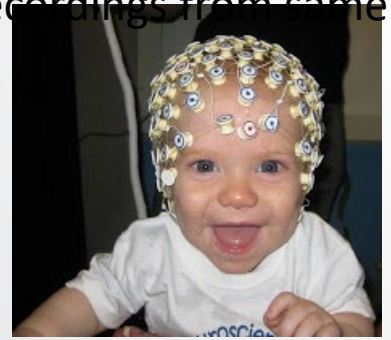


"Brain-on-a-Chip": Complex 2D model

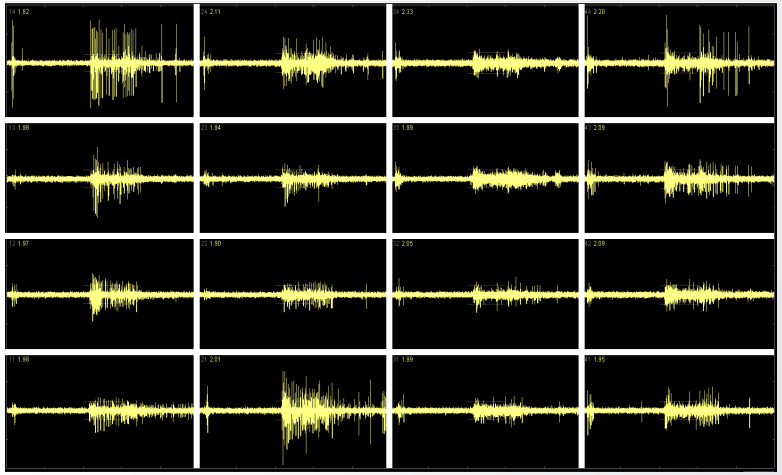
- Rat cortical neural networks
- Contains neurons & glia cells
- Spontaneous activity
- Develops rapidly in vitro
- Follow network development over time
- Integrates activity of multiple processes

Microelectrode Array Recording

- Planar microelectrodes are non-invasive
- Records electrical activity of any tissue type
- Repeated recordings from same sample

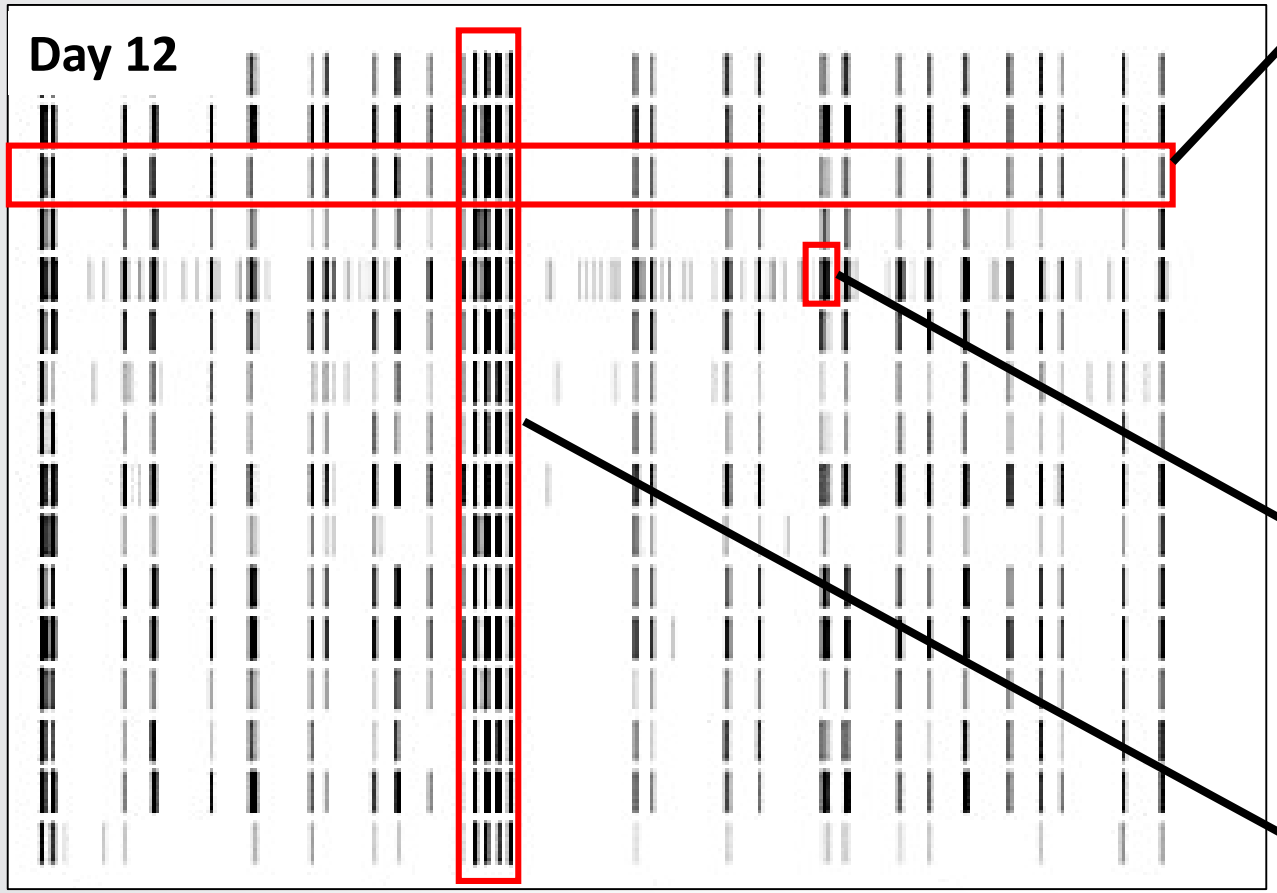


The electrical activity recorded by MEAs are the biological underpinnings of EEG recordings.



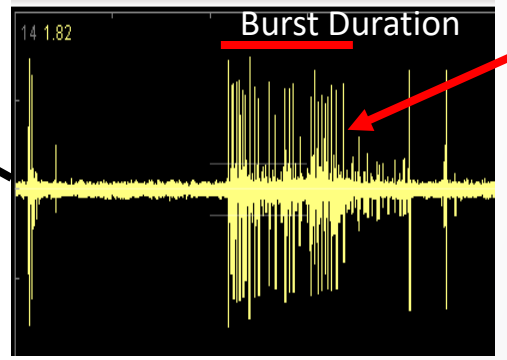
A snapshot in time of neural network activity in one well. Each box represents the electrical activity of neurons on 1 electrode in the array.

MEAs Measure Multiple Characteristics of Network Function



General Activity- overall rate of firing or bursting; measured on each electrode and averaged across the well.

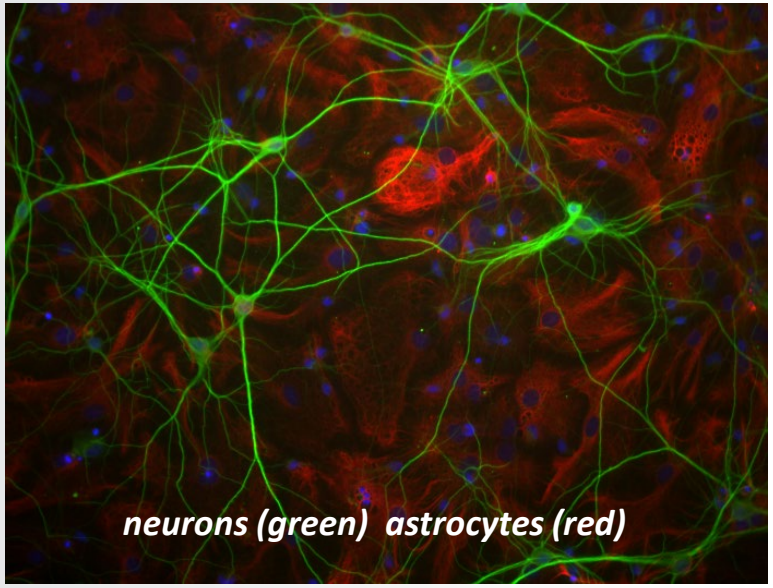
Bursting Structure- the length and number of events in a burst; measured on each electrode and averaged across the well.

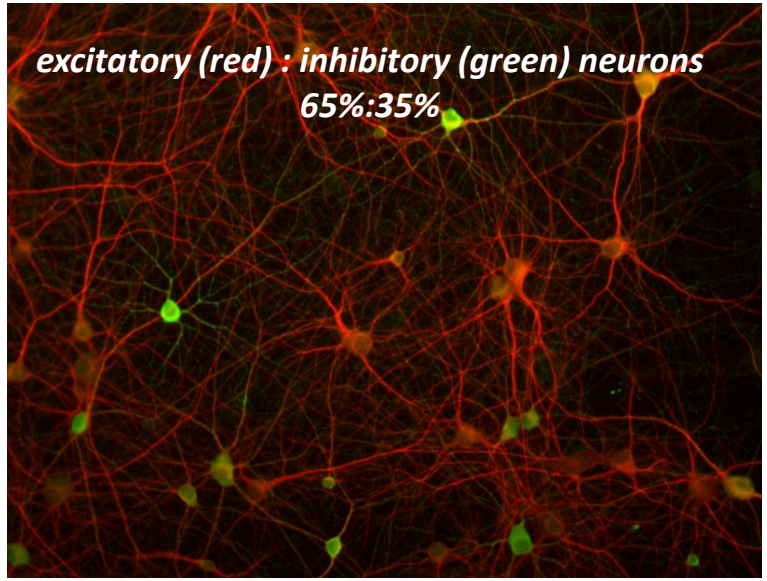


Number of Action Potential "Spikes"/burst

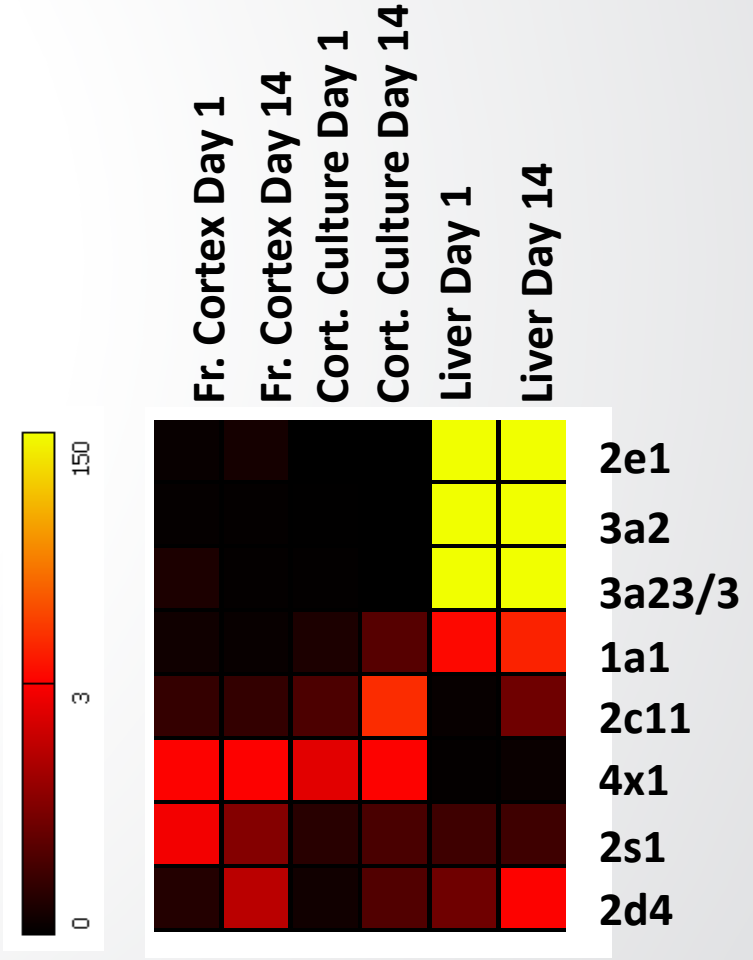
Connectivity- Communication of information across electrodes (Correlation coefficients, Network Spikes, Mutual Information); averaged for the well.

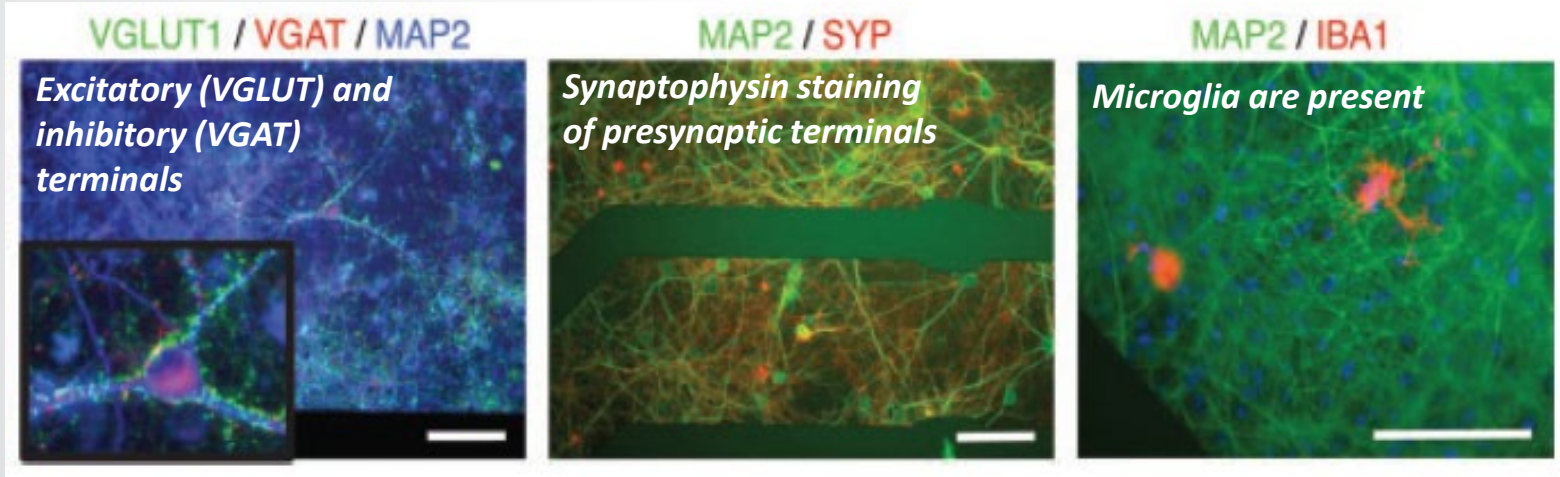
Primary Cultures of Cortical Neurons are Complex and Representative of *in vivo* Cortex



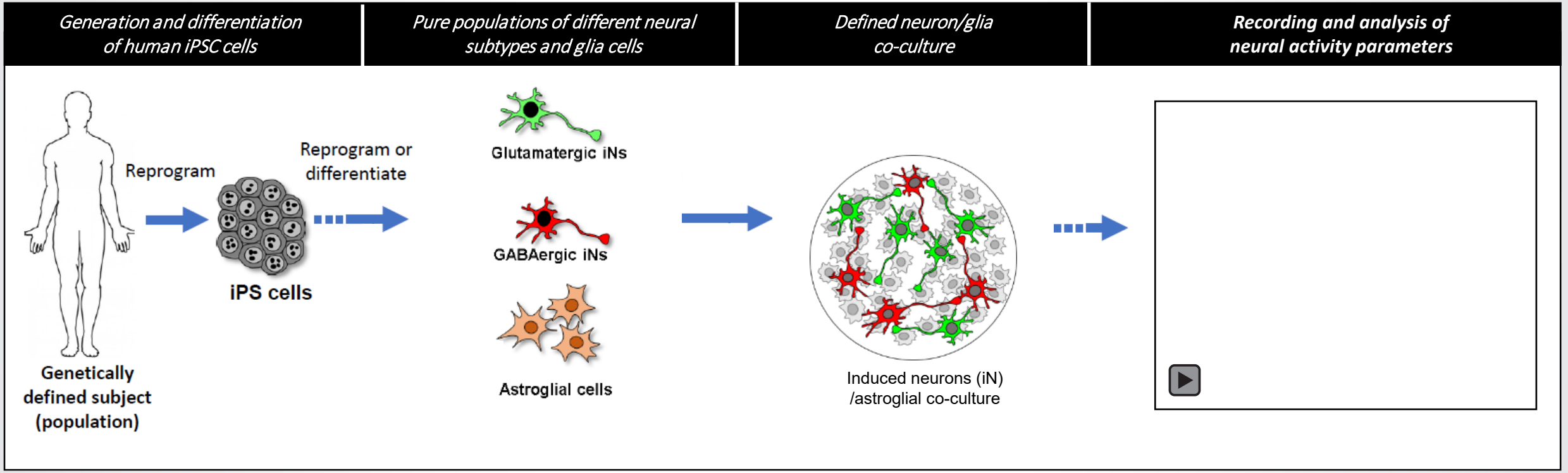


P450 expression is similar to Frontal Cortex, but lower than and different from the liver





SynFire® human iPSC-derived induced neuron (iN)/glial co-culture system



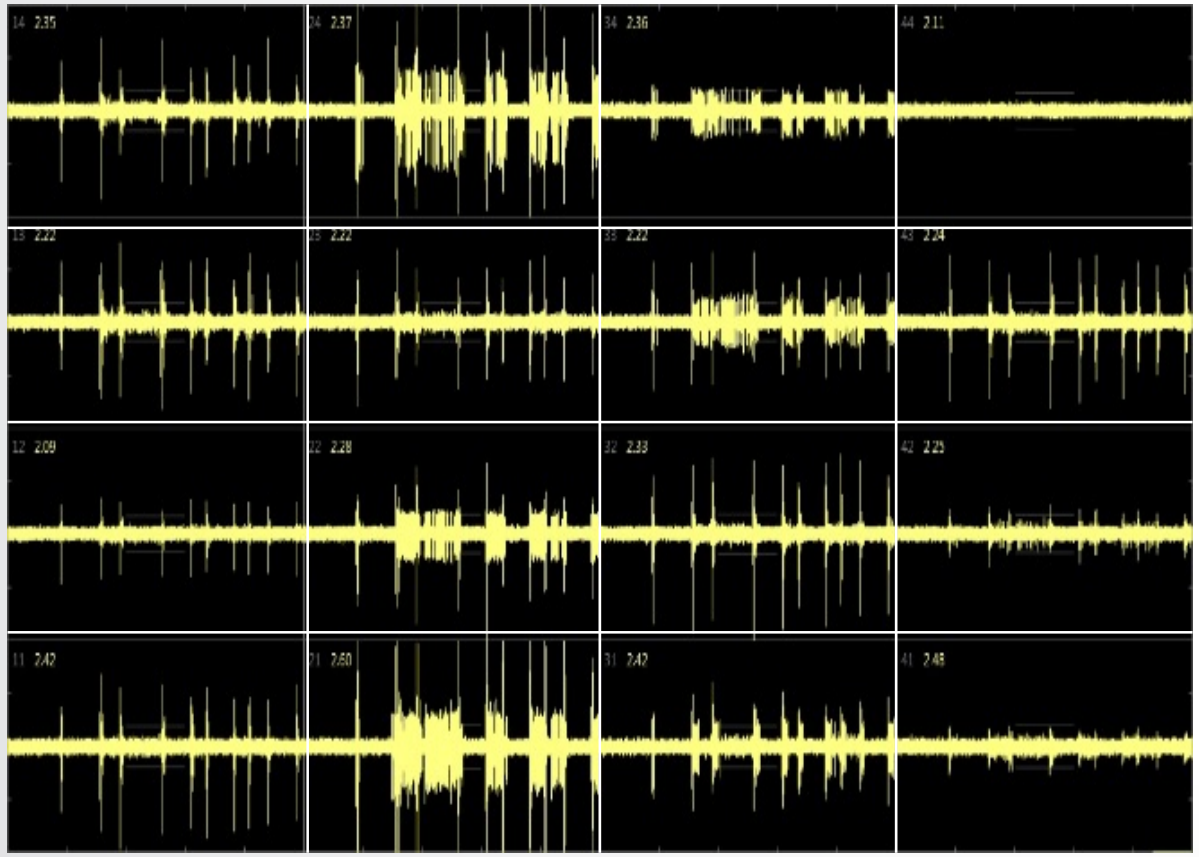
Synfire human iPSC-derived induced neurons (iNs) and glia

- Fast maturation and low variability
- Specified cell composition and reliable and robust readouts
- Cell-type specific modification for flexible assay design
- Rapid developing complex synchronized network activity

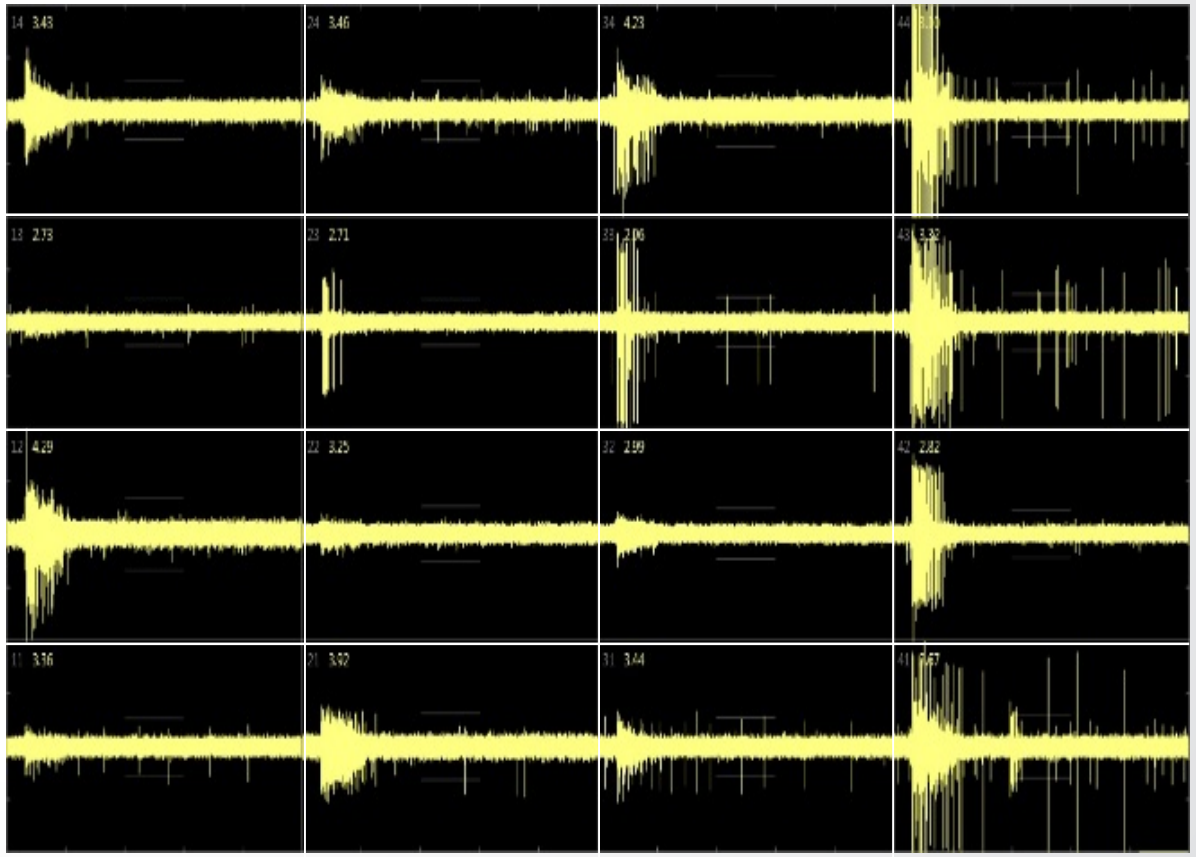
Slide Courtesy of NeuCyte

SynFire and Rodent Cortical Networks have Similar Phenotypic Profiles in ORD Labs

Rat Network, DIV 23

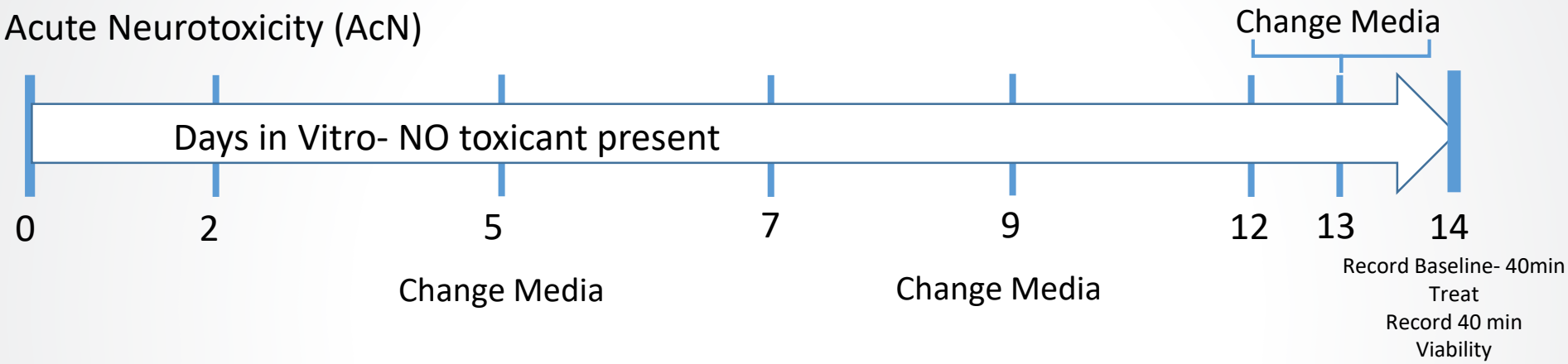


Human Network, DPP 37

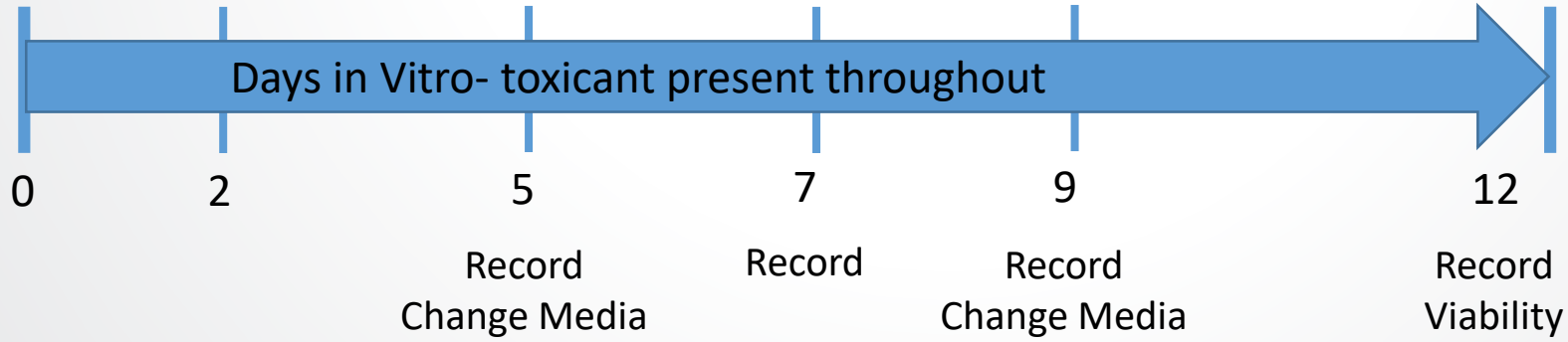


Testing chemicals for effects using MEAs: Two different assays

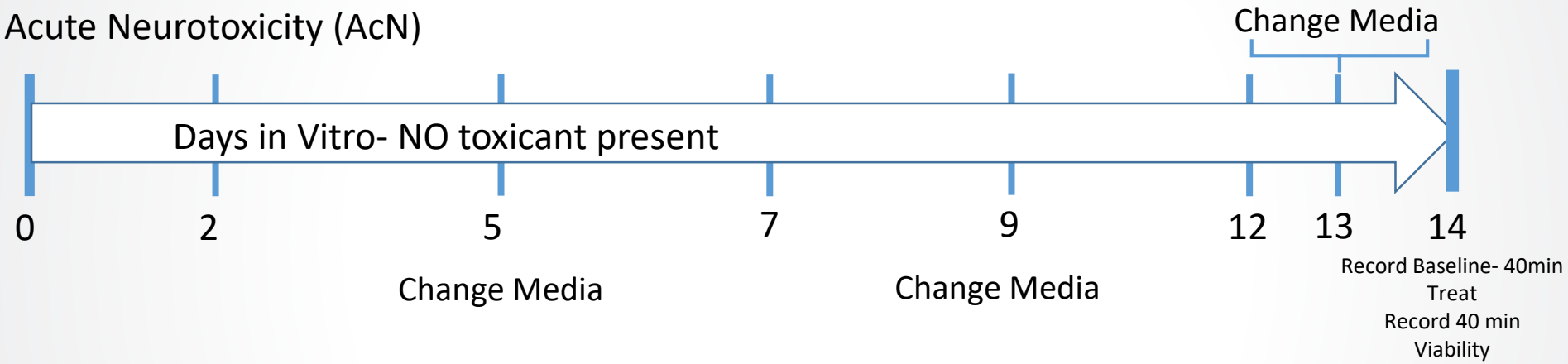
Acute Neurotoxicity (AcN)



Network Formation Assay (NFA)



Testing chemicals for acute effects on network function



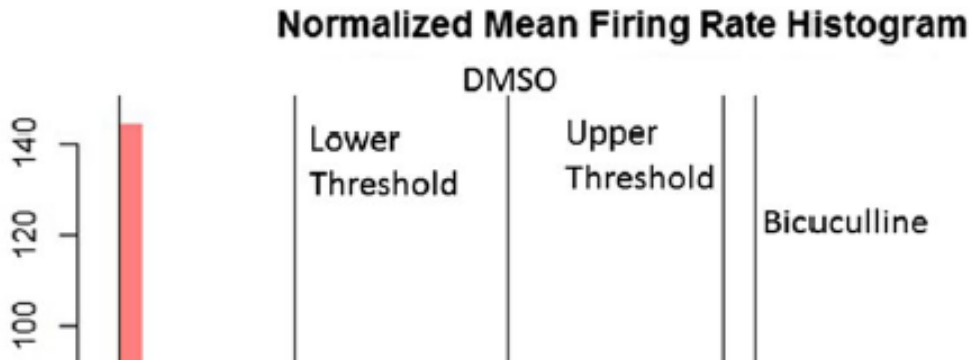
Progress to Date:

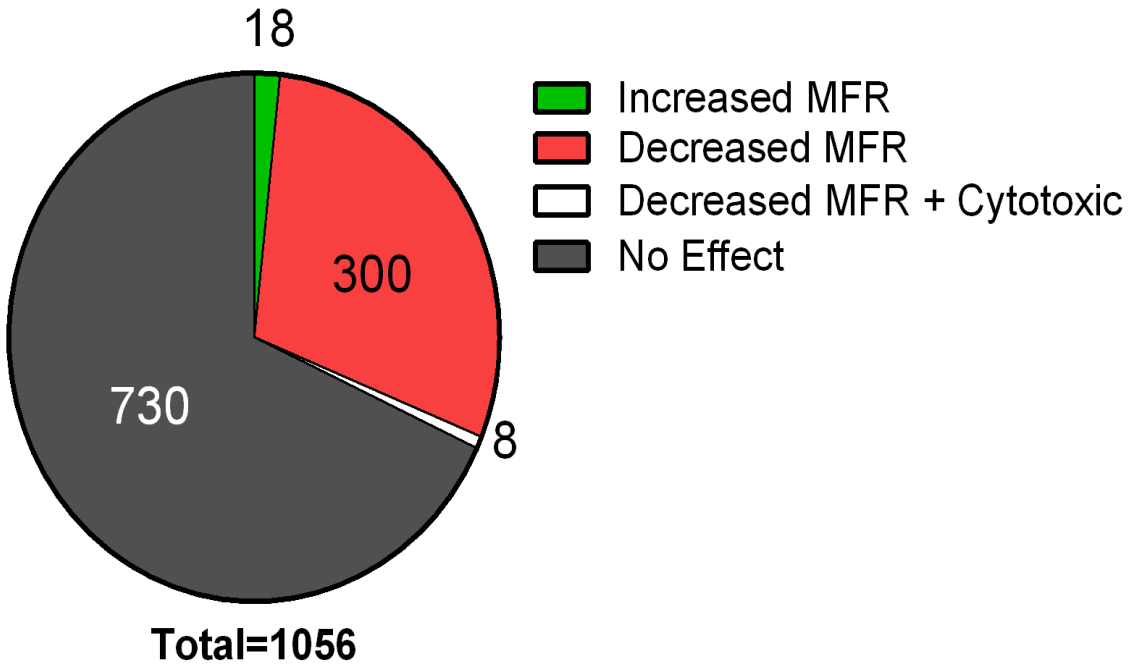
- **McConnell et al., 2012.**
  - **Proof of Concept-30 Chemicals**
- **Valdivia et al., 2014.**
  - **100 ToxCast Compounds**
- **Strickland et al., 2018**
  - **1055 ToxCast Phase I & II Compounds, single conc.**
- **Kosnik et al (2020)**
  - **384 Compounds from Strickland, Conc-Response**
- **Unpublished**
  - **74 EFSA/EPA Compounds**

= > 1100 Compounds

The Majority of ToxCast Compounds are Without Effect on Network Activity

Compound Effects on MFR





Compounds that Increased MFR

Organochlorines		Neonicotinoids
Aldrin	DDT	Nicotine
Endrin	DDE	Imidicloprid
Heptachlor	Lindane	Thiamethoxam
Heptachlor epoxide		

Compounds that Decreased MFR

Organochlorines	Mectins	Pyrethroids
Endosulfan	Abamectin	Allethrin
Kepone	Emamectin	Cypermethrin
Methoxychlor		Fenpropathrin
		Prallethrin
		Tetramethrin

Strickland et al., Archives of Toxicology. 2018. 92, 487-500.

Further characterization of neurotoxicity with MEAs

- Rescreen hits from single-concentration screen on MFR and other parameters of network function for concentration response
- Evaluate endpoint parameters to determine subset most informative in characterizing neuroactivity
- Characterize active compounds based on fingerprints of neuroactivity and structure

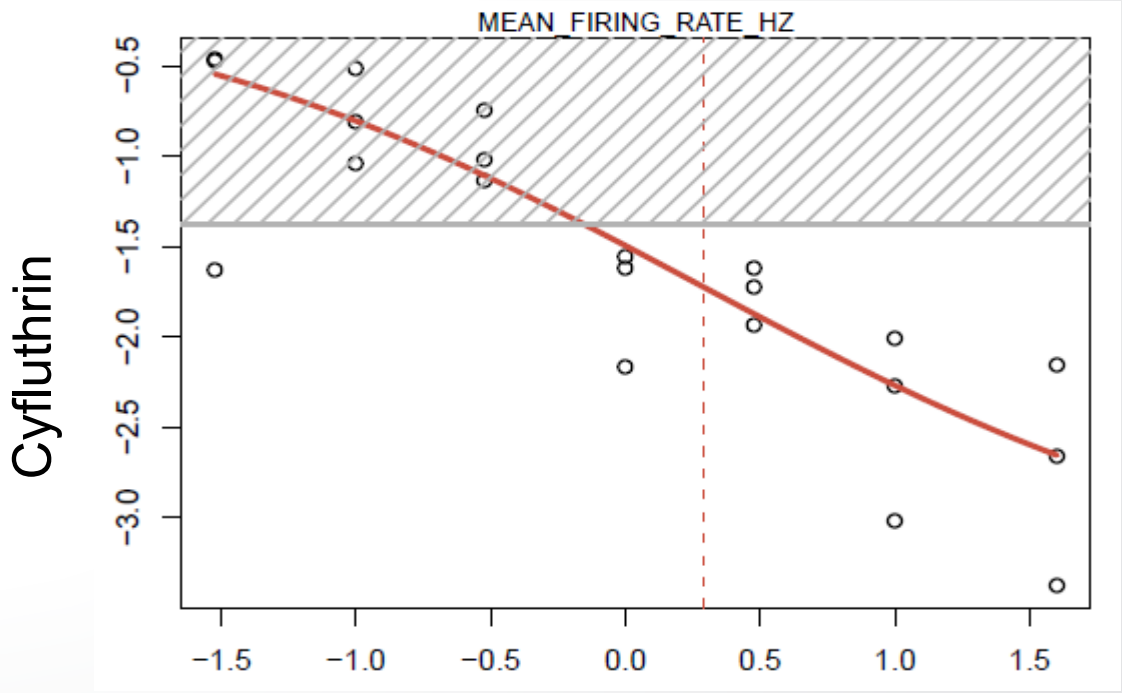
MEA methods

- 384 compounds (222 active in single concentration screen)
- Concentration range, 7 concentrations of 0.03-40 µM
- 43 parameters (endpoints) including MFR
- Baseline activity recorded 40 minutes before addition compound
- Activity recorded as additional 40 minutes with compound
- Alamar Blue for cytotoxicity

Identification of active compounds with ToxCast Analysis Pipeline (tcpl)

- 384 compounds with 43 endpoints run through tcpl
- Response= dose-baseline
- Hits: at least one median response greater than 3*bmad (baseline median absolute deviation)
- 5,423 total hits across 374 compounds and 43 endpoints

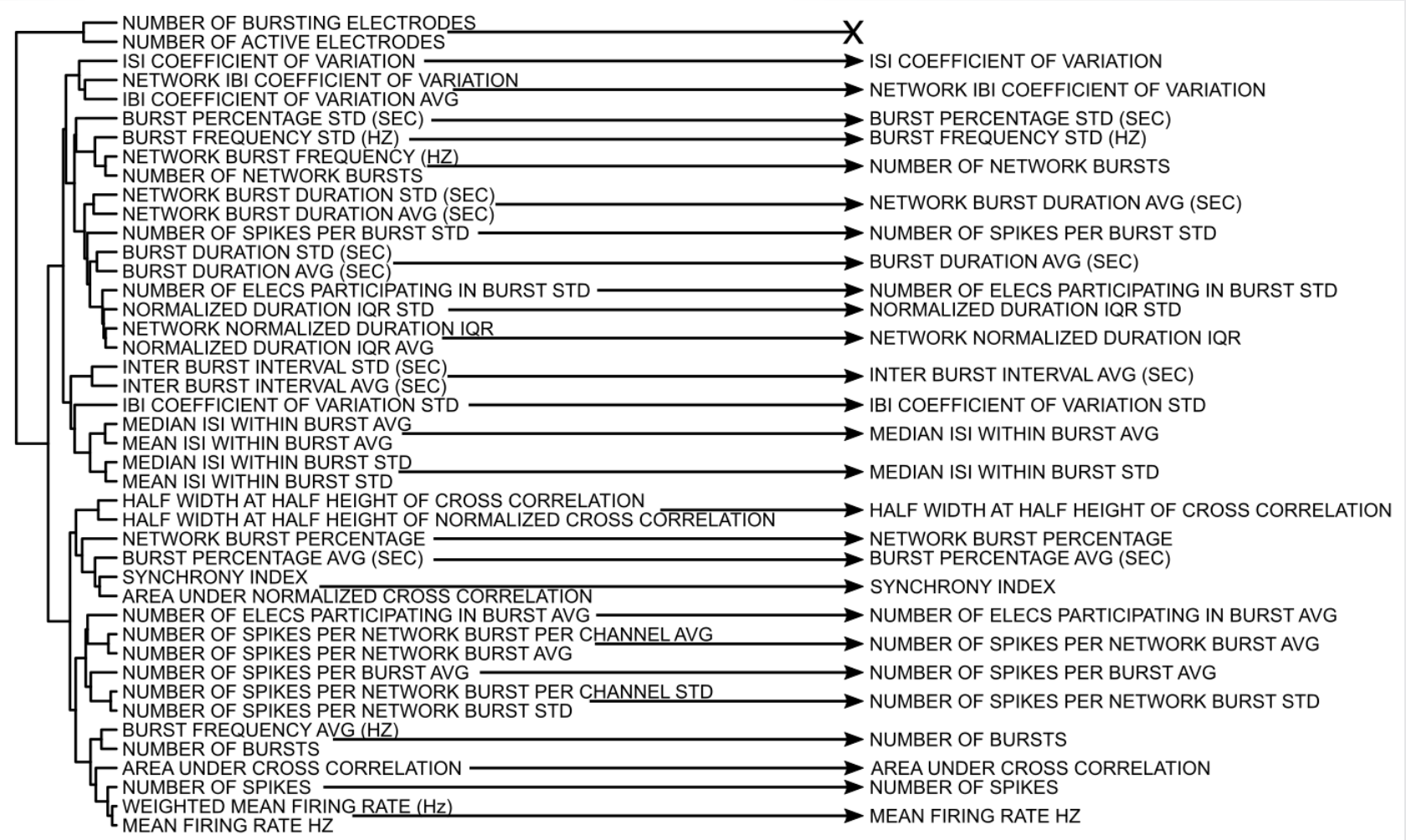
Concentration-response curve for mean firing rate MEA endpoint. Grey box is 3*BMAD cutoff for activity, dashed red line is AC50.



Kosnik et al., 2020

Feature Reduction Approaches

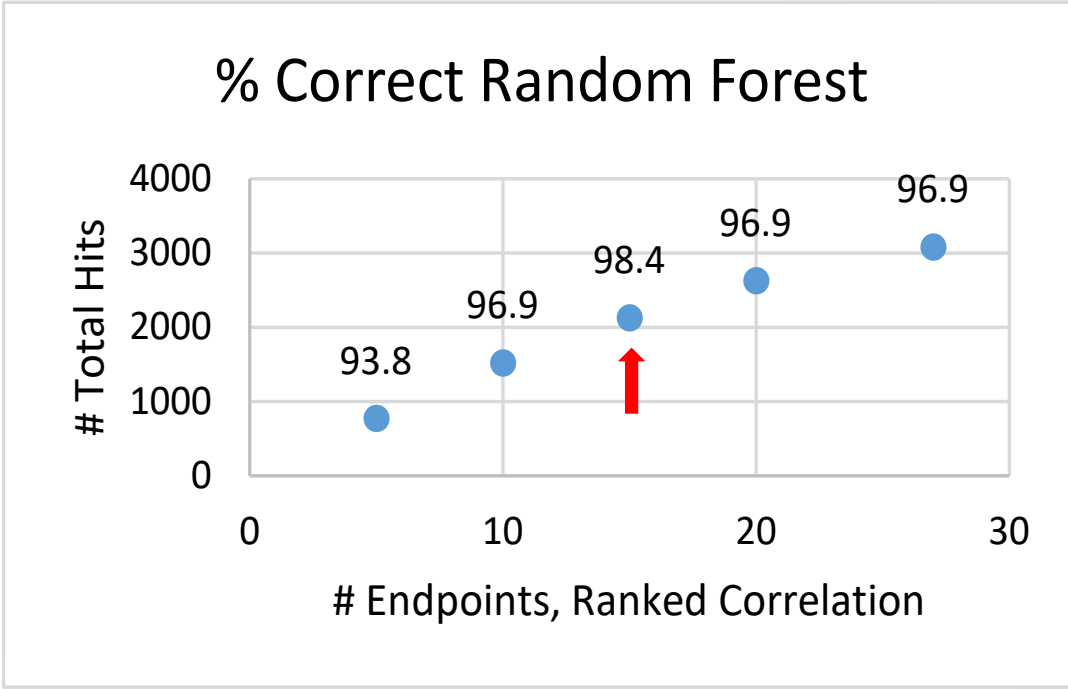
- Clustered 43 endpoints using distance function adjusted to include differences between:
  - Chemical-endpoint AC50s
  - Winning AIC models (Hill vs Gain-loss)
- Reduced endpoint list to 27 endpoints by removing most redundant endpoint



Kosnik et al., 2020

Additional feature reduction steps

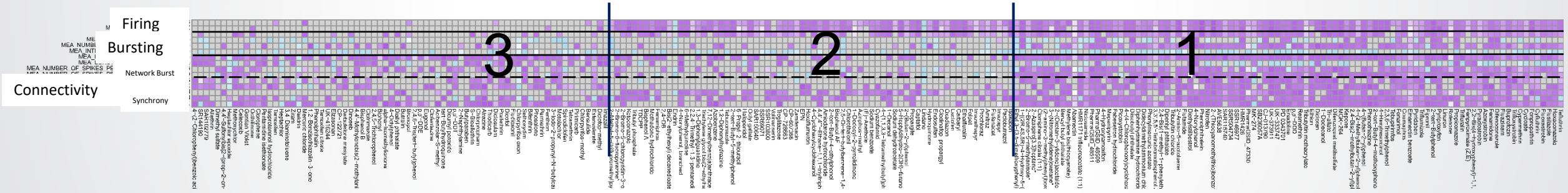
- Developed expert-curated list of neuroactive and non-neuroactive compounds in dataset
  - 41 neuroactive
  - 32 non-neuroactive
- Used machine learning to identify and rank endpoints that best distinguish between neuroactive and non-neuroactive chemicals
- From ranked list, reduced number of endpoints to
  - 20
  - 15
  - 10
  - 5
- Repeated process 3x with the active chemical set
- Reduced to those active in at least 1, 2, or 3 endpoints



Kosnik et al., 2020

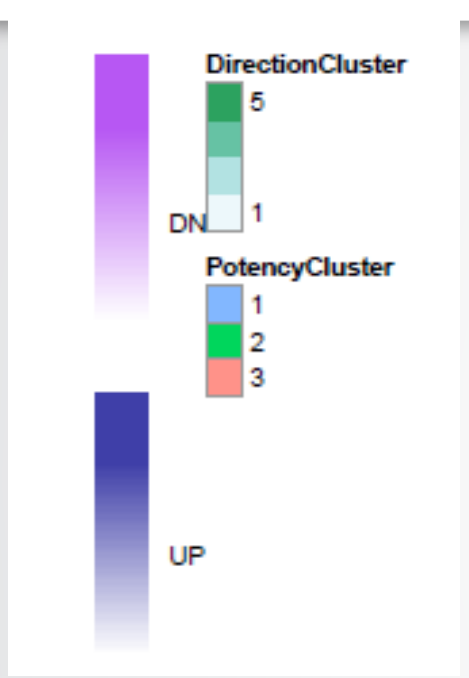
Characterization of chemical activity patterns

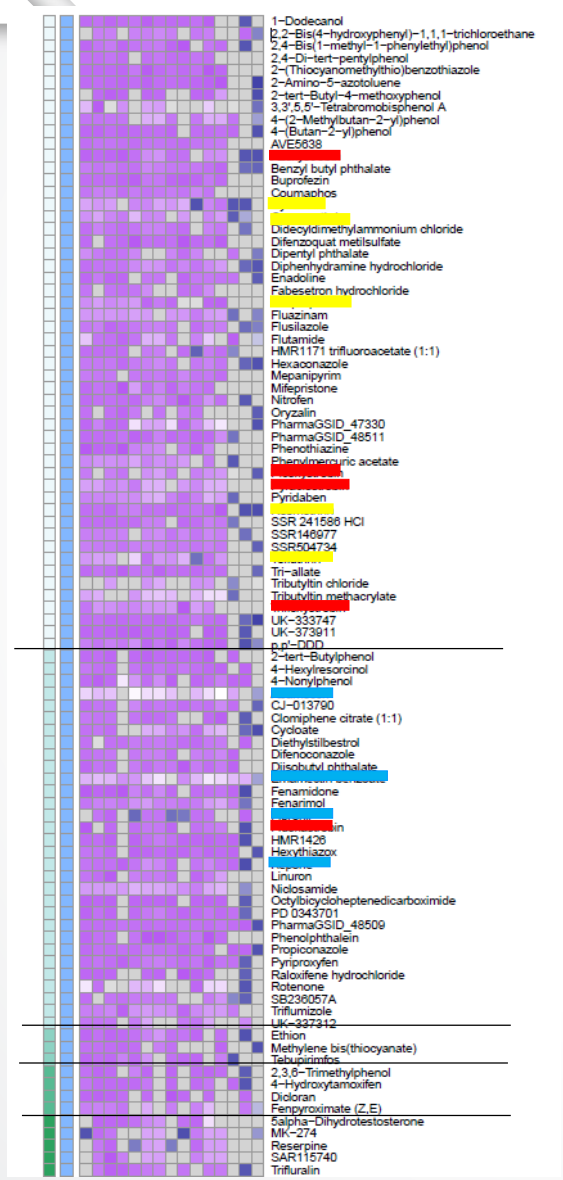
- Analyzed differing patterns of endpoint activity among chemical groups using k-means clustering
- Determined that three clusters best classified active compounds

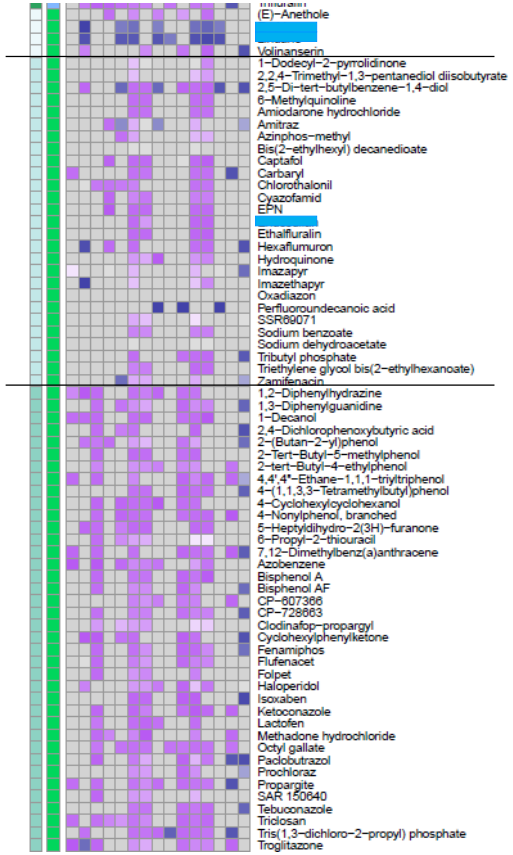


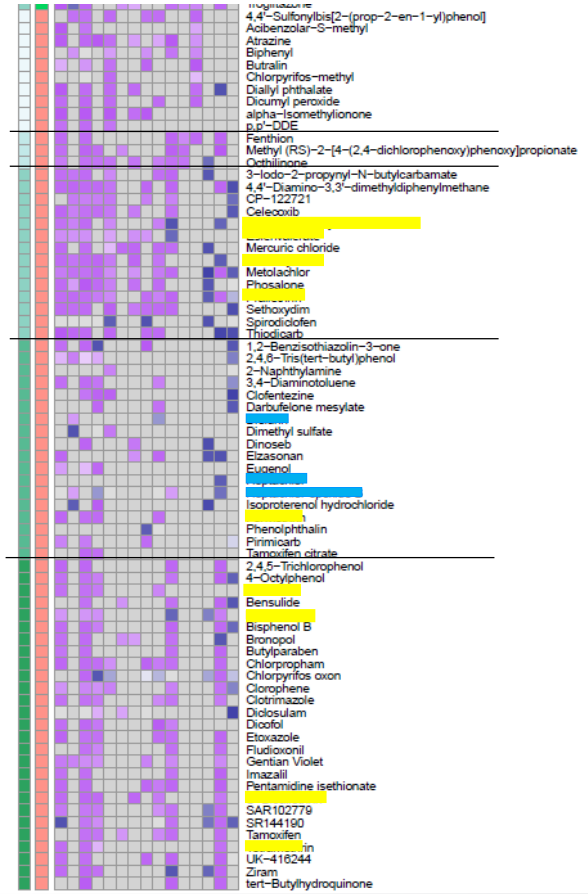
Kosnik et al., 2020

Clustering separates compounds with similar modes of action









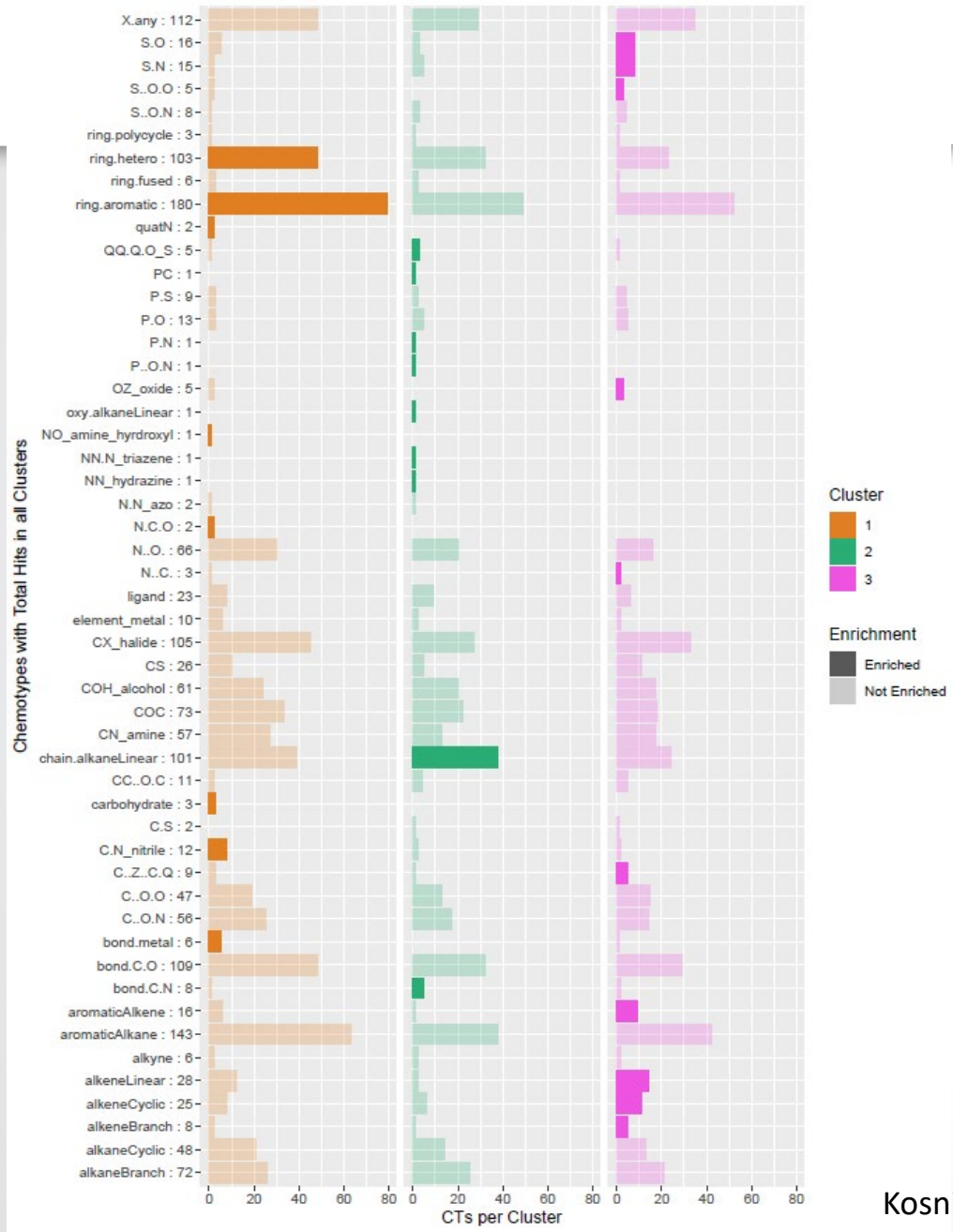
VGSC Modulator

$GABA_A$ (Ant)agonist

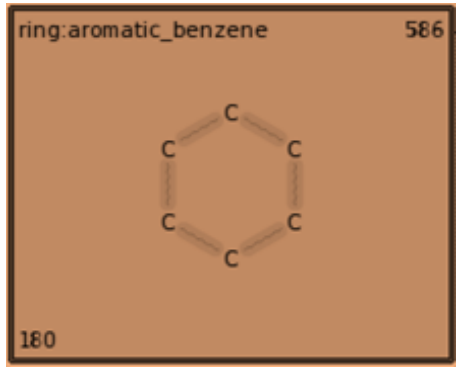
Strobilurin fungicide

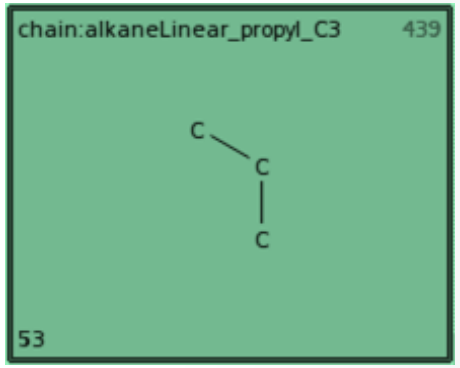
Kosnik et al., 2020

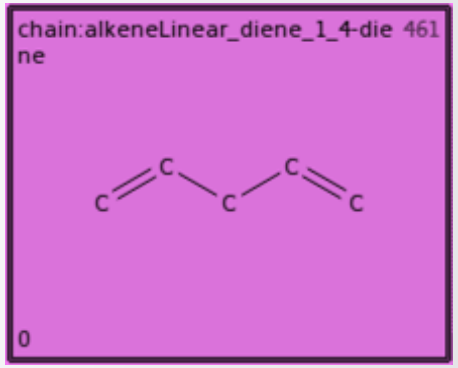
Chemotype Enrichment



- Different chemotype patterns are enriched in each cluster
  - Cluster 1= 8 chemotypes
  - Cluster 2= 9 chemotypes
  - Cluster 3= 10 chemotypes





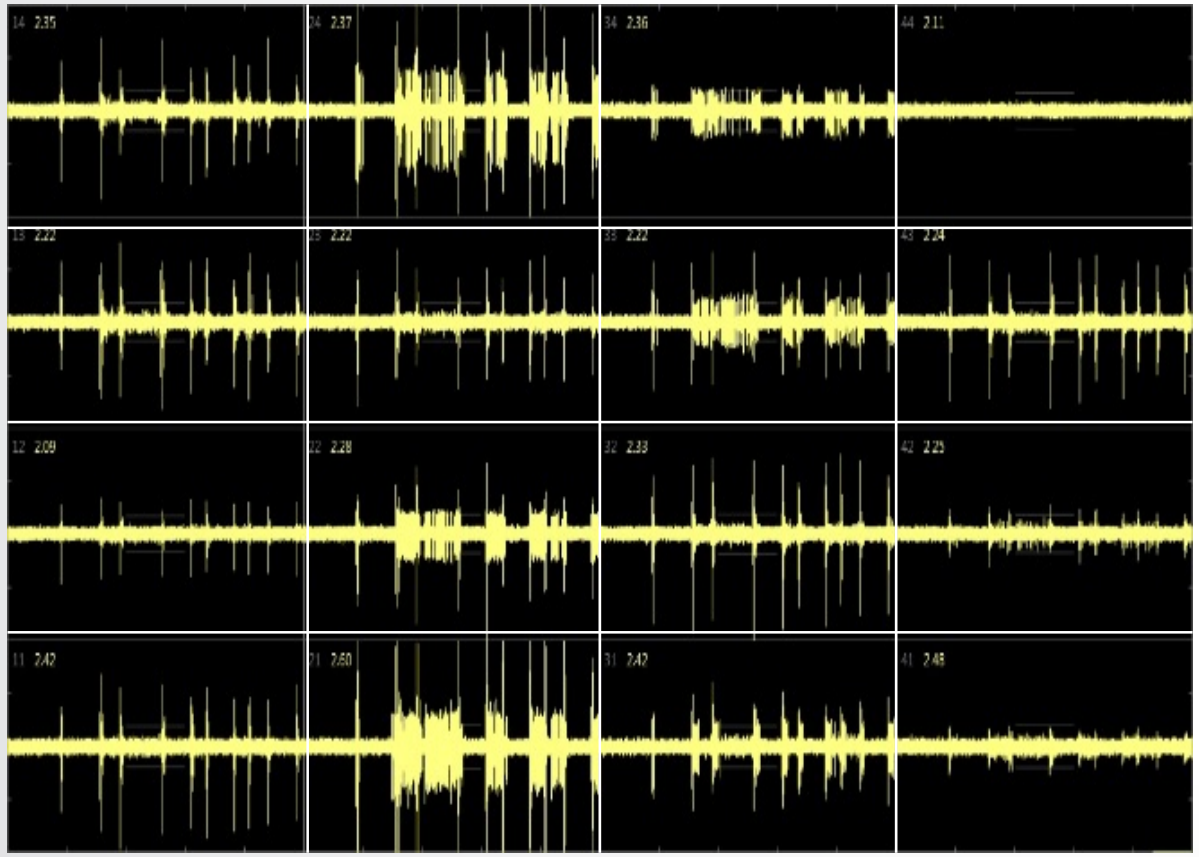


Sub-structural chemical features contribute to the different activity fingerprints within k-means clusters

Kosnik et al., 2020

Comparison of compound effects in rat and human networks

Rat Network, DIV 23



Human Network, DPP 37

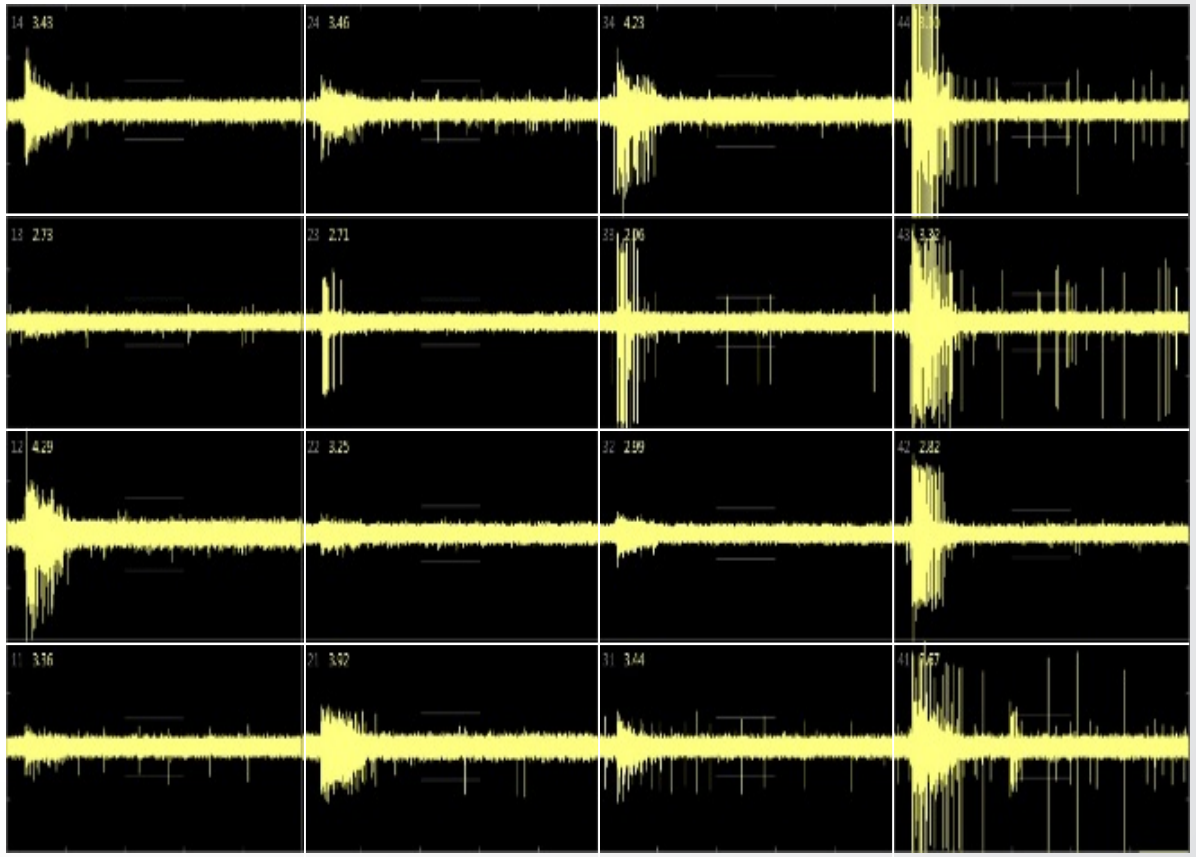
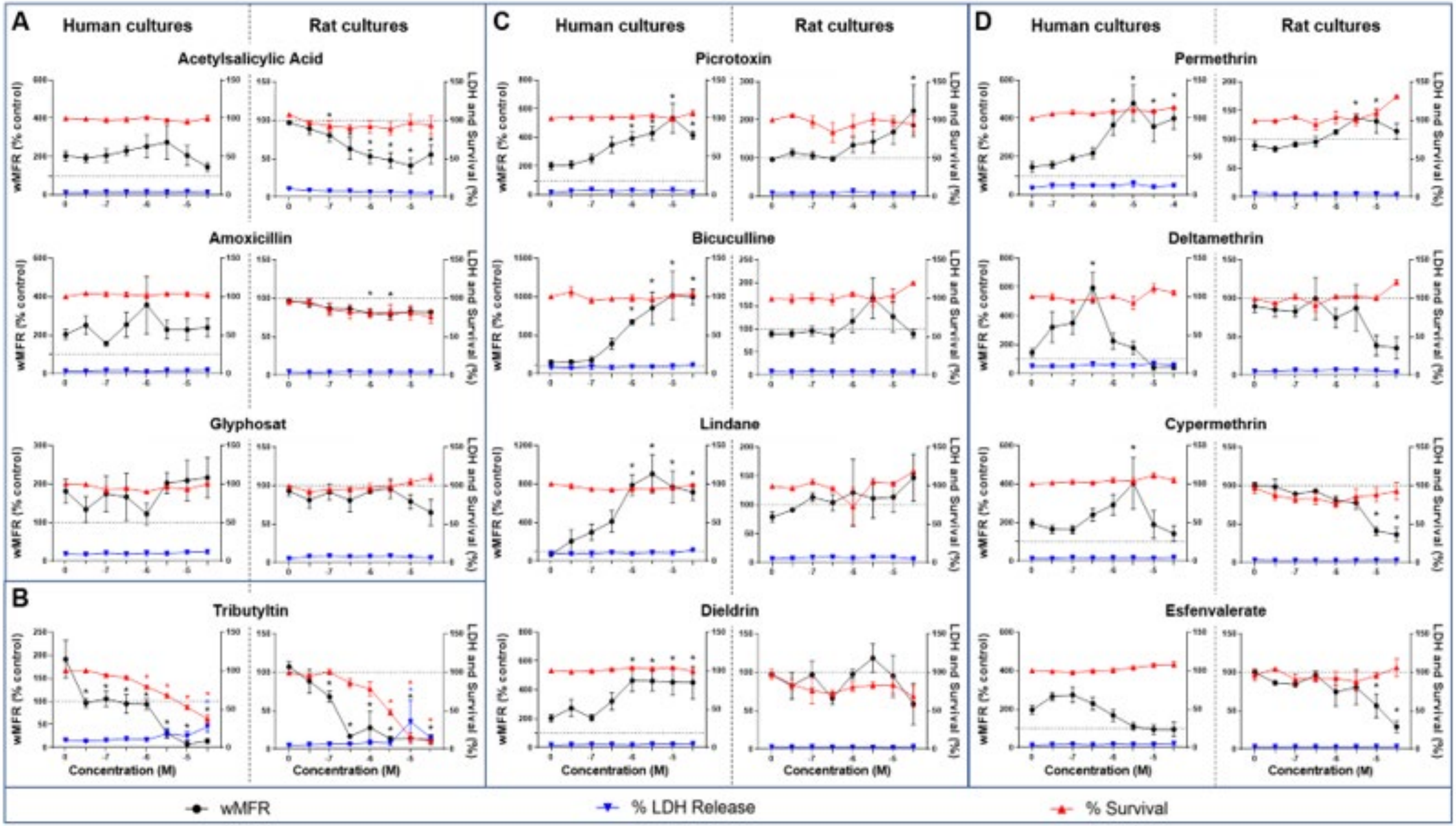


Table 1: Chemical compounds used for neurotoxicity testing on MEAs

Compound	CAS #	DTXS ID	Class	Effect	Solvent	Purity
Amoxicillin	26787-78-0	DTXSID303704	penicillin-class antibiotic	negative control	DMSO	≥90%
Salicylic acid	69-72-7	DTXSID7026368	non-steroidal anti-inflammatory drug	negative control	DMSO	≥99%
Glyphosate	38641-94-0	DTXSID0034649	organophosphorus herbicide	negative control	water	96%
Bicuculline	485-49-4	DTXSID3042687	isoquinoline alkaloid	$GABA_A$ antagonists	DMSO/ethanol	≥99%
Picrotoxin	124-87-8	DTXSID7045605	convulsant alkaloid	$GABA_A$ antagonists	DMSO	98%
Lindane	58-89-9	DTXSID2020686	organochloride insecticide	$GABA_A$ antagonists	ethanol	99%
Dieldrin	60-57-1	DTXSID9020453	organochloride insecticide	$GABA_A$ antagonists	DMSO	≥95%
Permethrin	52645-53-1	DTXSID8022292	type I pyrethroid insecticide	modulation of Voltage-sensitive sodium channel (VSSCs) kinetics	DMSO/ethanol	≥91%
Deltamethrin	52918-63-5	DTXSID8020381	type II pyrethroid insecticide	prolonged modulation of VSSCs kinetics	DMSO/ethanol	≥98%
Cypermethrin	52315-07-8	DTXSID1023998	type II pyrethroid insecticide	prolonged modulation of VSSCs kinetics	DMSO/ethanol	≥98%
Esfenvalerate	66230-04-4	DTXSID4032667	Type I/II pyrethroid insecticide	intermediate modulation of VSSCs kinetics	DMSO/ethanol	98.5%
Tributyltin	56-36-0	DTXSID7043950	organotin biocide	oxidative stress	DMSO	96%

Saavedra et al., In revision



Saavedra et al., In revision

Conclusions for Neurotoxicity

- Unsupervised analysis of parameters demonstrated that measurements of Firing, Bursting and Network Communication are important endpoints that separate neuroactive from inactive compounds.

- Active compounds cluster around their effects on these parameters, and that clustering corresponds with their chemical structure.

- Further consideration of direction of effects allows for better separation of compounds.

- Rodent and human IPS-derived neurons produce comparable results for a small set of compounds.

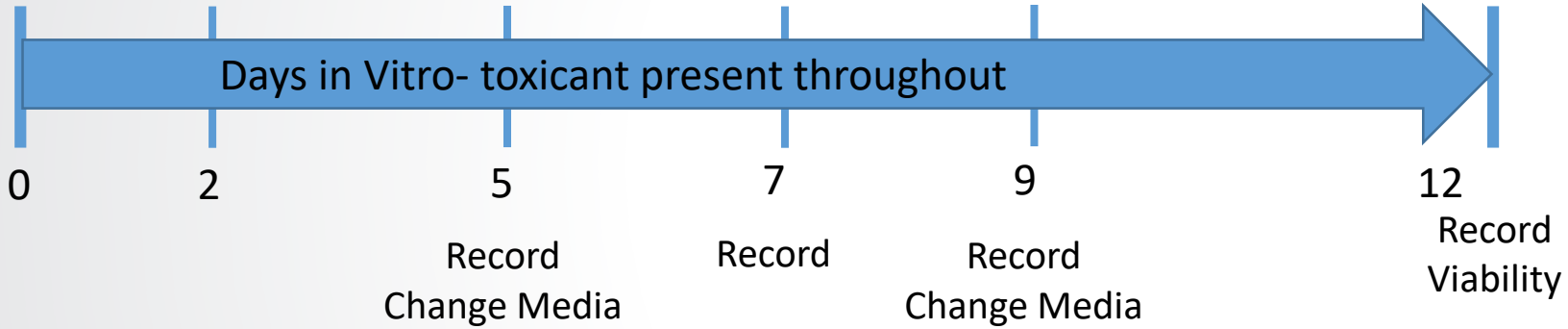
Challenges:

- Of the compounds tested here, there were not enough representative compounds for many pharmacological actions to assess separation. Many other compounds have unknown modes of action.

- Temporal changes in the data are not considered, and may also provide useful information.

Chemical Effects on Network Formation

Network Formation Assay (NFA)

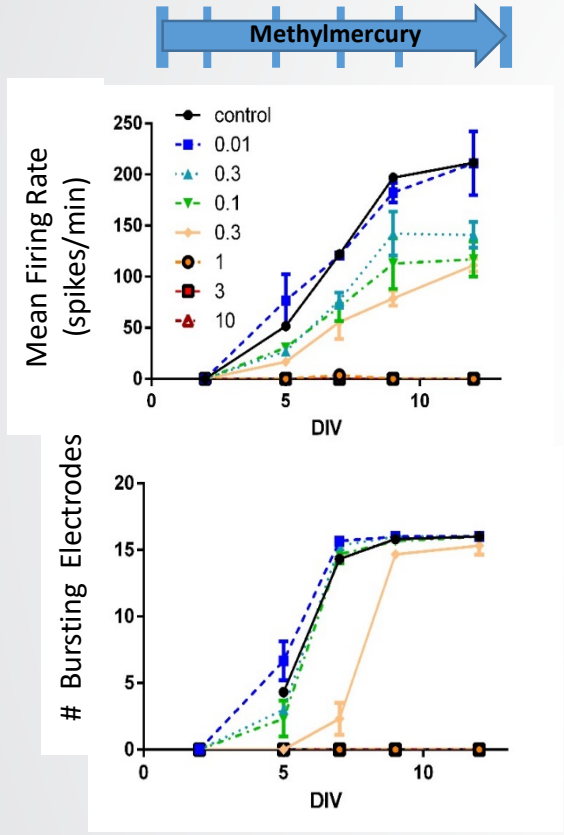


Tested to Date:

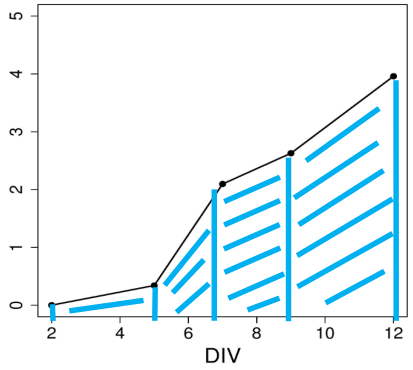
- **Brown et al., ToxSci. 2016.**
  - **Proof of Concept-6 Chemicals**
- **Frank et al., ToxSci. 2017.**
  - **DNT Reference Set-60 Chemicals**
  - **ToxCast/Uncharacterized- 20 Compounds**
- **Shafer et al., ToxSci. 2019**
  - **96 ToxCast Compounds**
  - **~40 NTP Compounds**
- **Unpublished**
  - **27 Organophosphates**
  - **75 PFAS Compounds**
  - **61 EFSA/EPA Compounds**

~300 compounds

Testing Chemicals for Effects on Neural Network Formation: Data Analysis in Brief

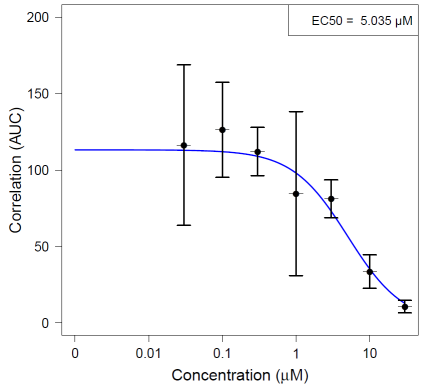


Determine Area Under the Curve for each dose (8) and parameter (17)

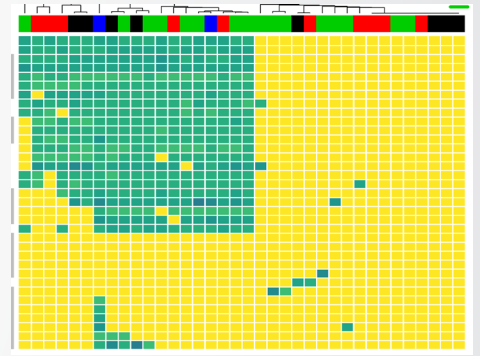


tcpl

Determine concentration-response

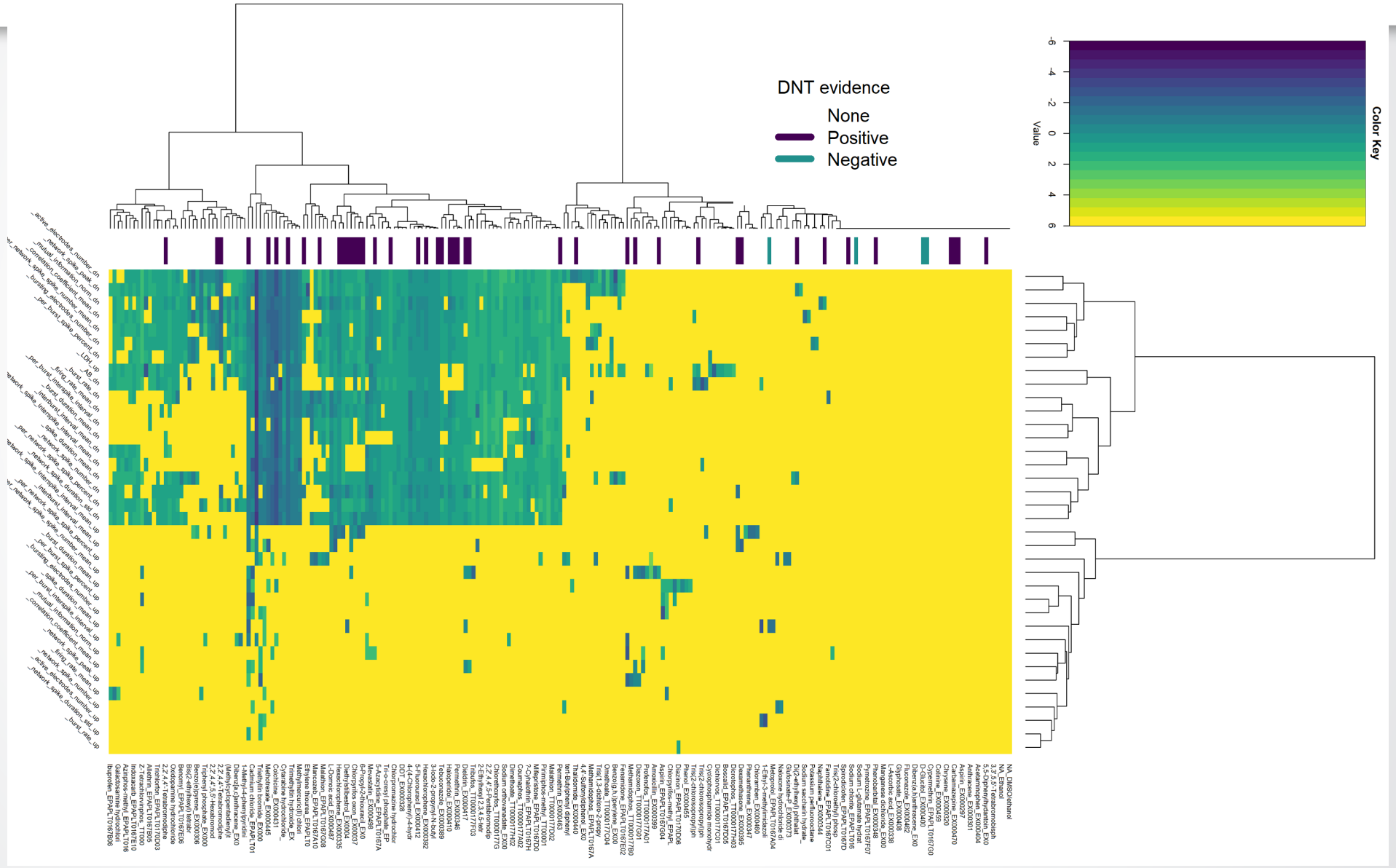


Generate comparisons of potency for many chemicals and endpoints

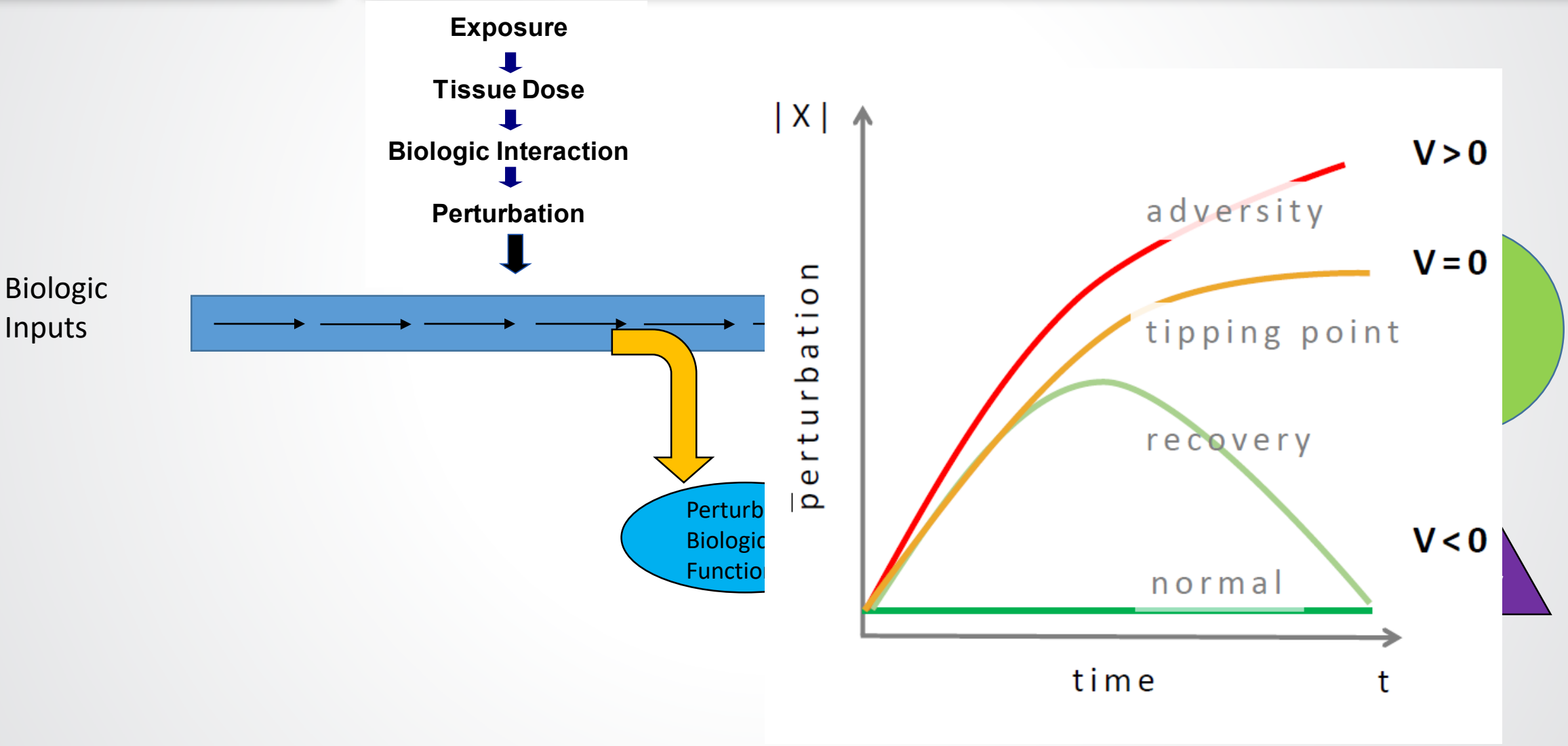


A snapshot of compound effects on network development

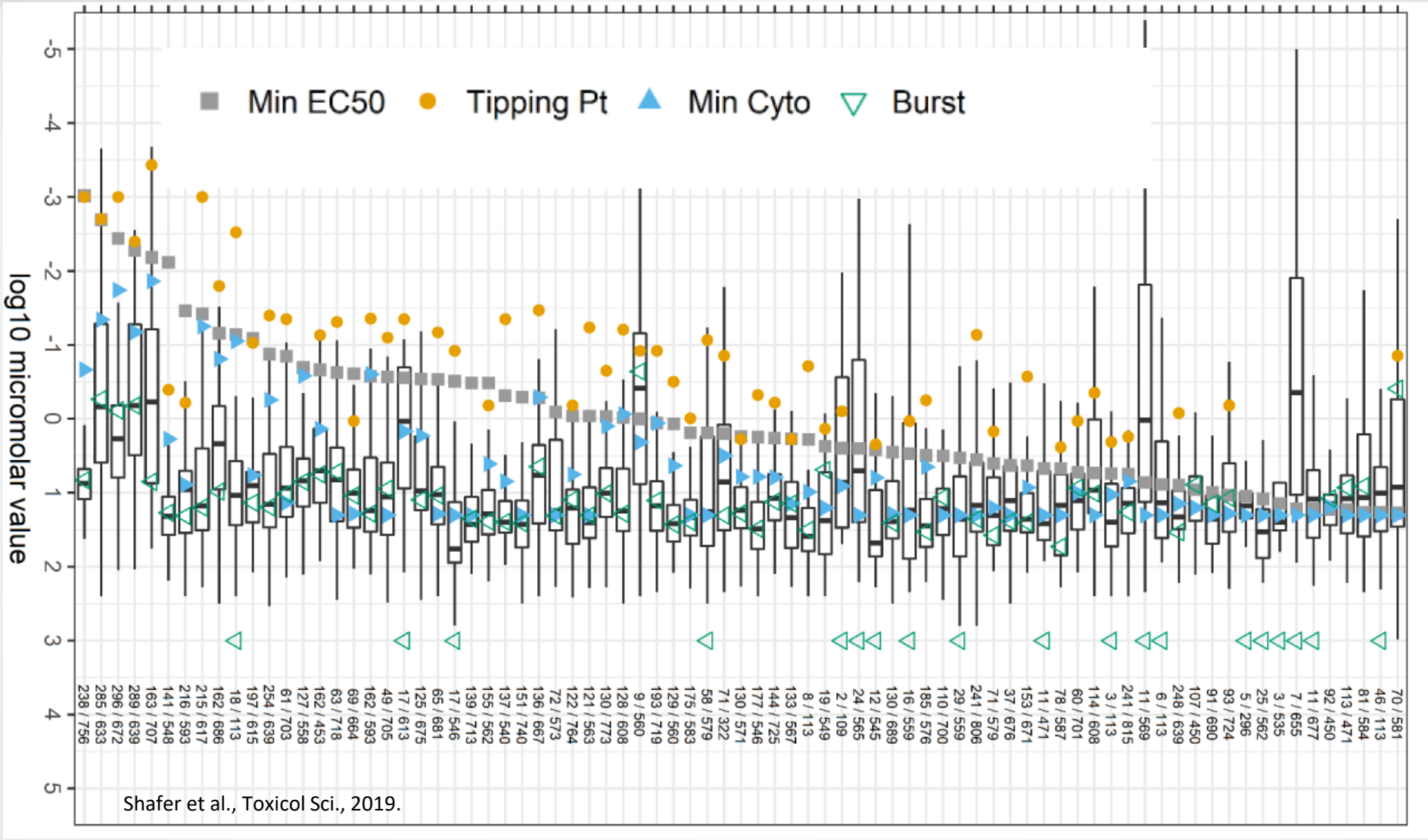
- A few main clusters; “on” and “off” behavior?
- Ability to distinguish negatives
- Cannot expect that one assay domain would identify all DNT positive chemicals.



The Concept of Toxicological "Tipping Points"

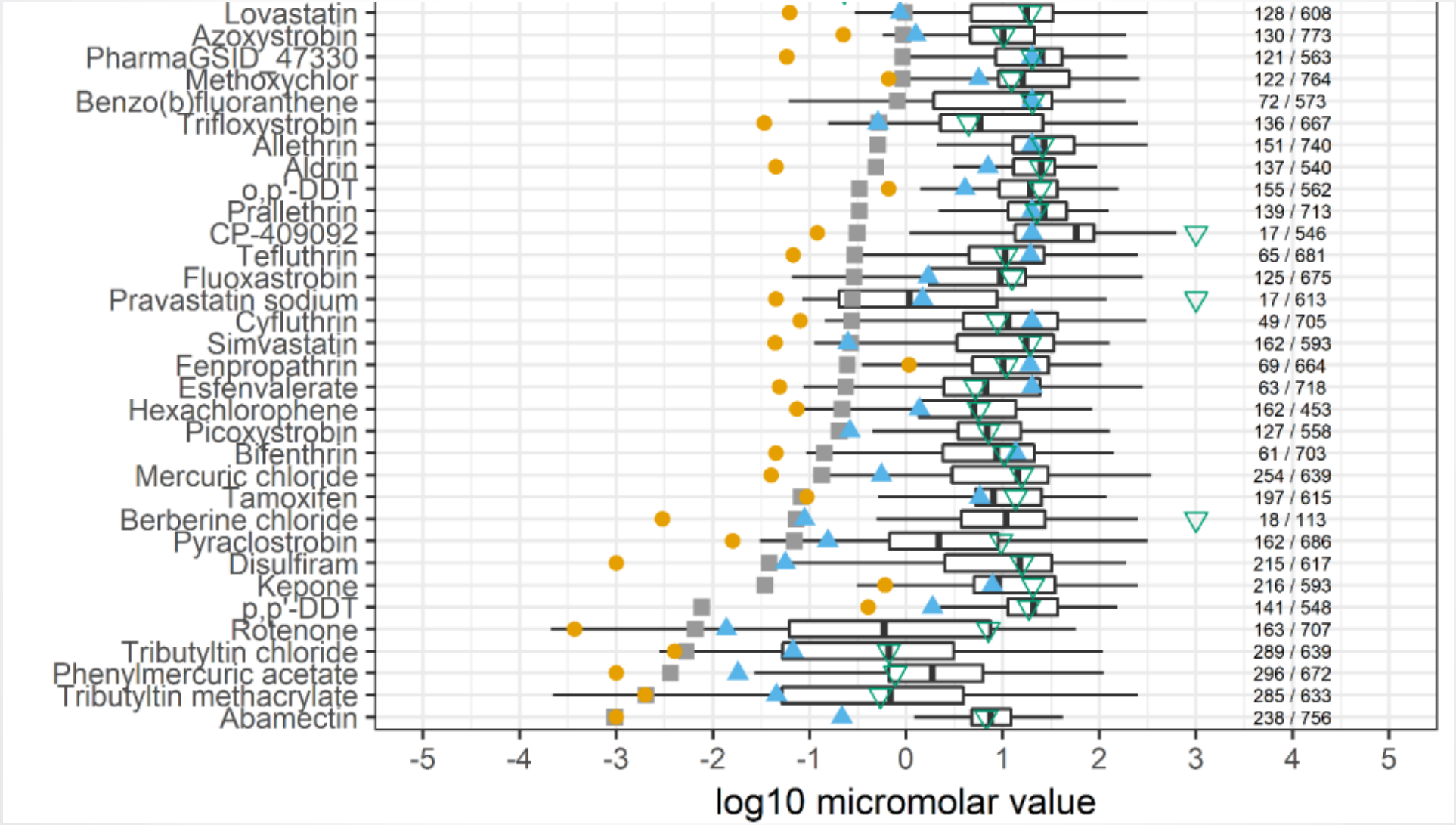


The MEA_NFA covers different biology than other ToxCast Assays

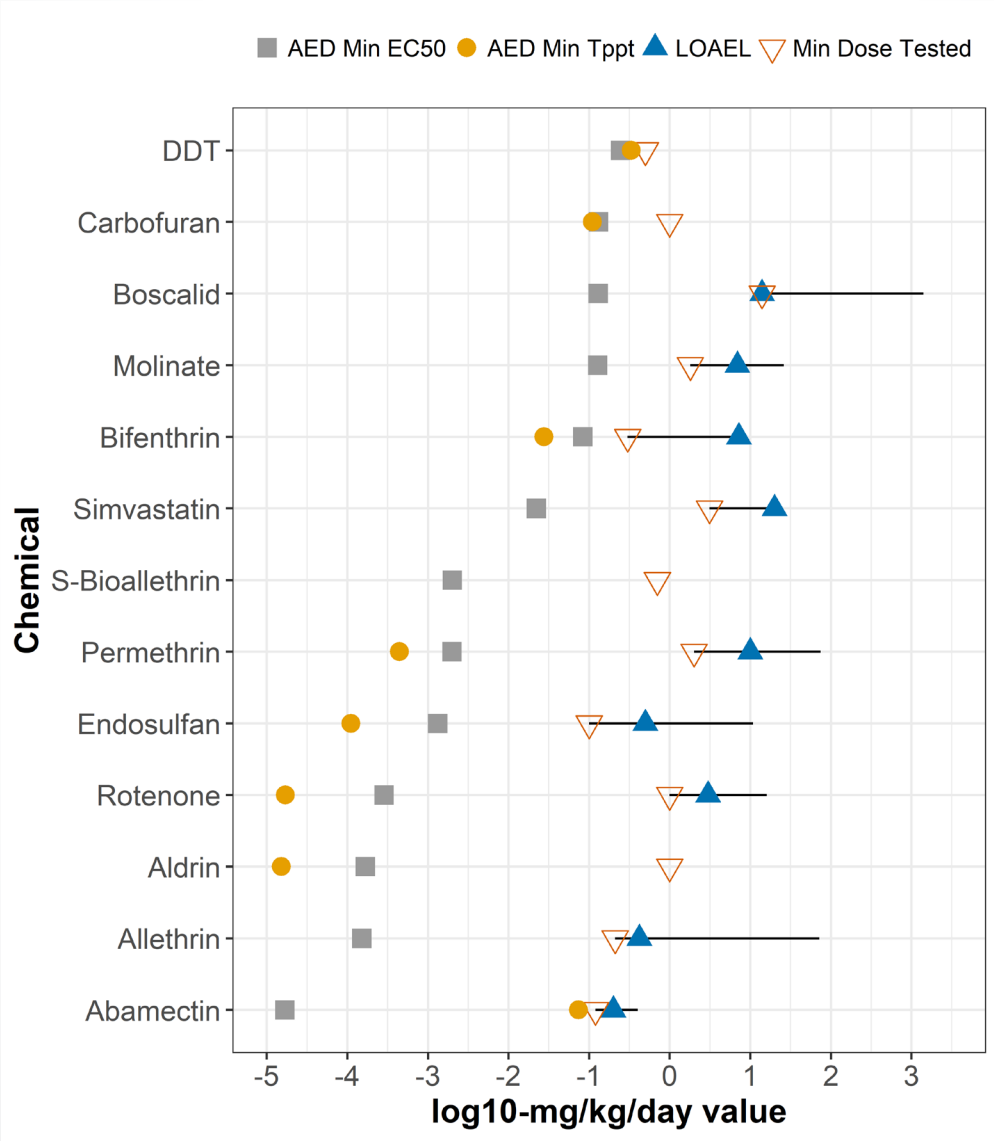


Shafer et al., Toxicol Sci., 2019.

The MEA_NFA covers different biology than other ToxCast Assays

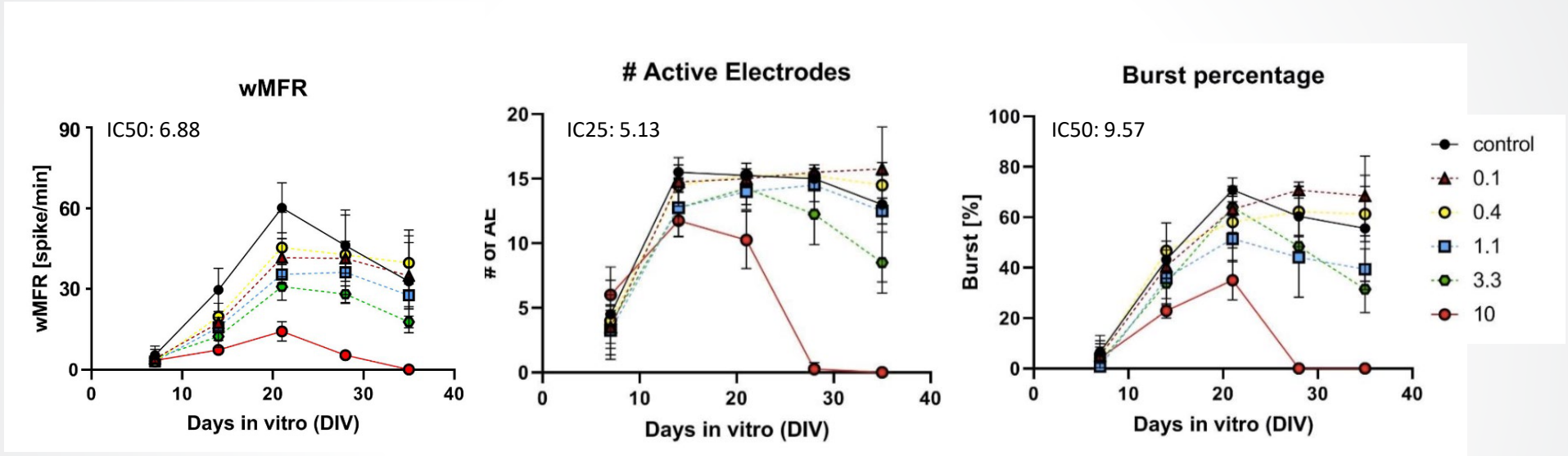


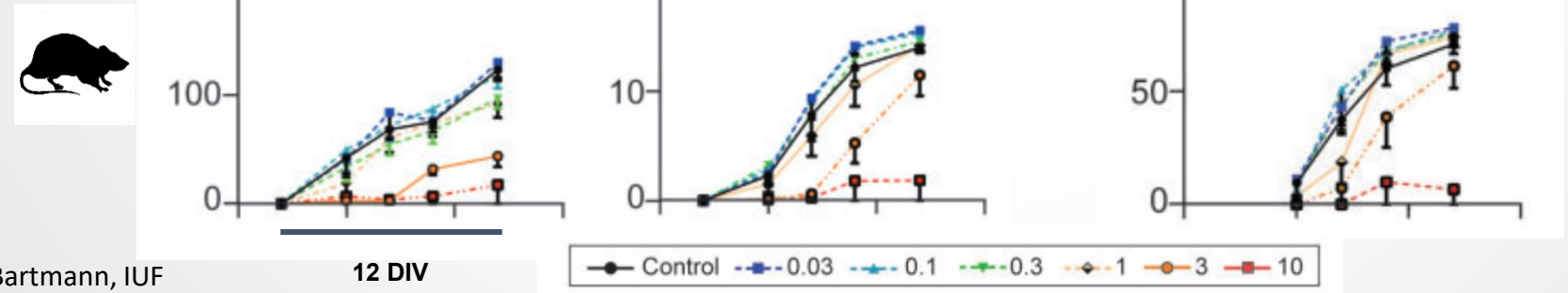
In vitro to *in vivo* Extrapolation indicates that MEA_NFA values are relevant



Shafer et al., Toxicol Sci., 2019

An Assay Based on Human Cells is Under Development





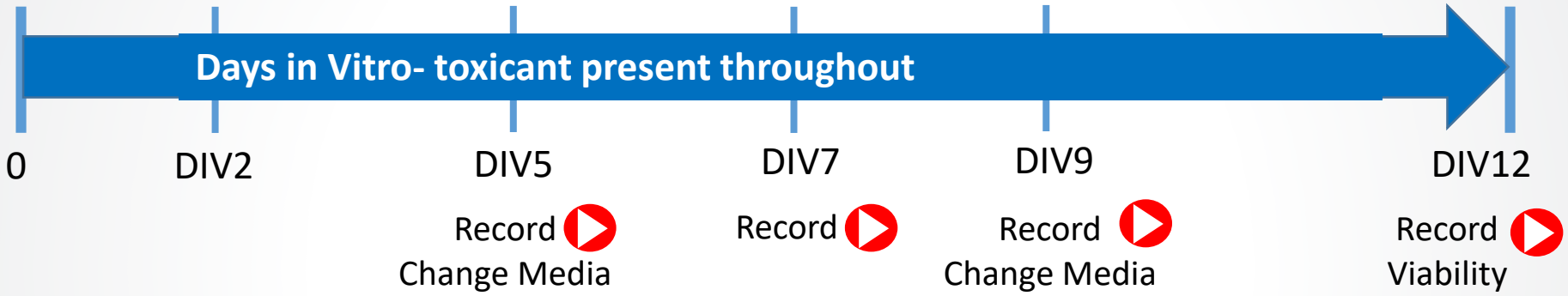
Courtesy of Kristina Bartmann, IUF

DIV: days in vitro
IC: inhibitory concentration

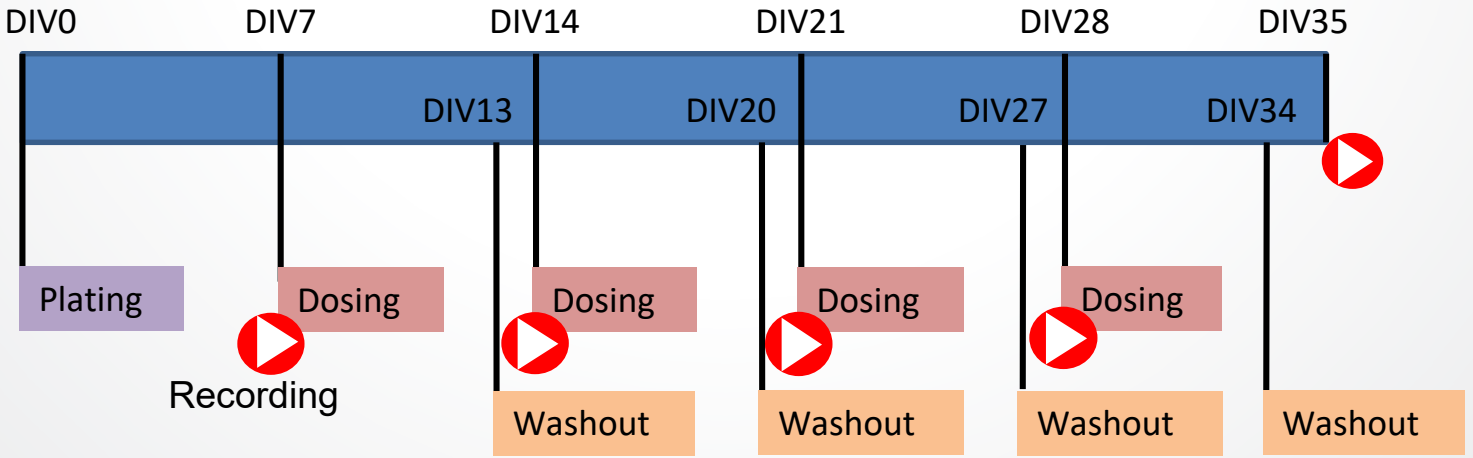
Brown *et al.* 2016
Shafer *et al.* 2019

Comparison between rat and human network formation assays

Rat Network Formation Assay (NFA)



Human Network Formation Assay (NFA-SynFire Neurons)



Summary of Network Formation

- The MEA_NFA is a sensitive measure for evaluating compound effects on the development of neural networks.
- This assay covers a biological space that is not well-represented by assays currently in ToxCast
- Following IVIVE, the concentrations at which effects are observed in the NFA occur at or below those causing in vivo DNT effects.
- Early data indicates that a NFA using human IPS-derived neurons is feasible.

Challenges:

- A stronger link between in vitro effects on network formation and in vivo alterations in structure/function would reduce the uncertainty around use of MEA_NFA data for regulatory decisions.
- Human networks, while feasible, take longer to develop and will be more expensive to use than rodent neurons.

Adverse Outcome Pathway Development

Well established AOPs involving Network Formation may reduce the uncertainty in using data from the MEA_NFA

While several of the few DNT-relevant AOPs in the AOPWiki include alterations in network function as a key event, overall there are few established AOPs linked to DNT

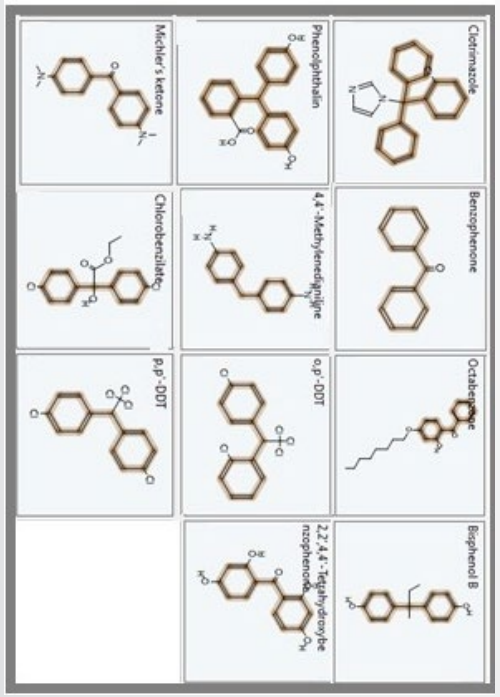
https://aopwiki.org/aops/15

High-throughput assays for DNT provide information for Adverse Outcome Pathway Development

Toxicant

Chemical Property Profile

→ "Chemotyping"



Molecular Initiating Event

? (many postulated, few confirmed)

→ **Target-based Screening** (known MIEs)

HTP Molecular assays (ToxCast)
ion channels
AChE
neurotransmitter receptors
growth factor receptors
transcription factors
kinases
…

Cellular Responses

Key Events
proliferation
differentiation
migration
neurite growth
synaptogenesis

Organ Responses

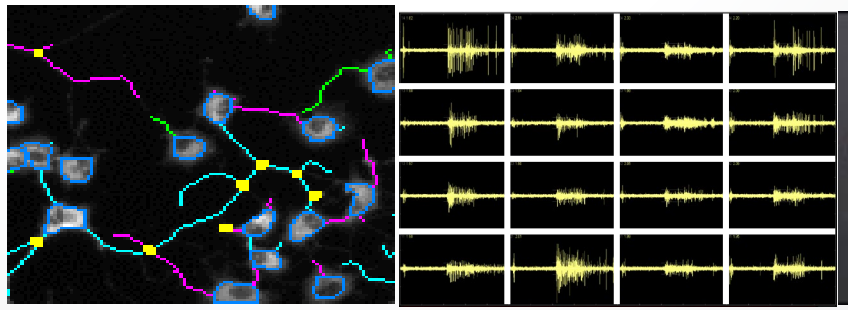
Nervous System
Δ connectivity (morphology, neurochemistry, physiology)

Individual Responses

behavior
cognition

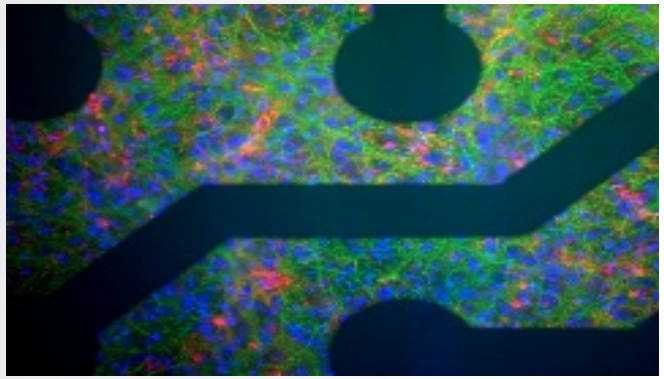
→ **Phenotypic Screening** in physiologically-relevant models (MIE not assumed)

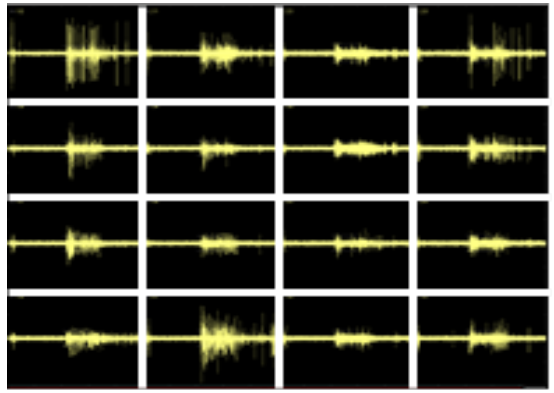
In Vitro Approaches

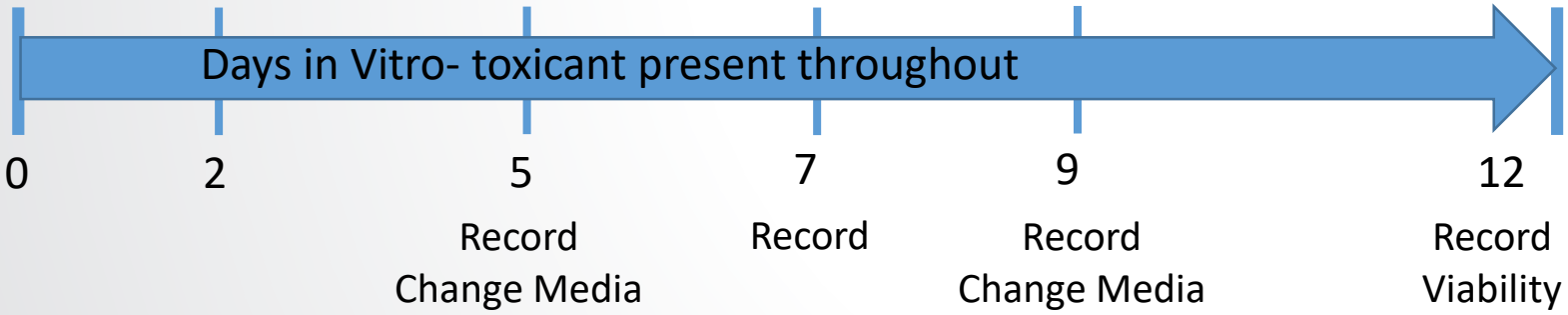


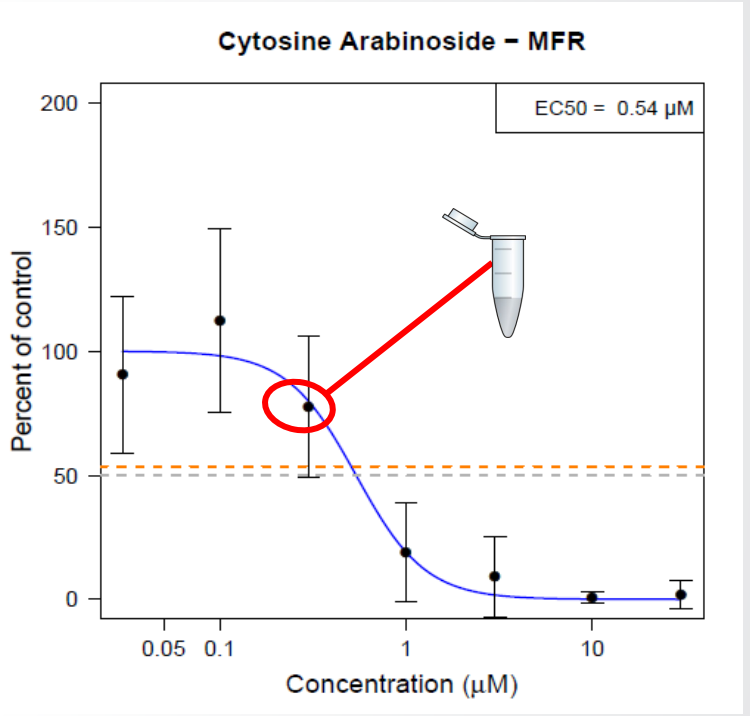
Behavior

Application of Transcriptomics and Metabolomics to *in vitro* DNT assays for AOP development

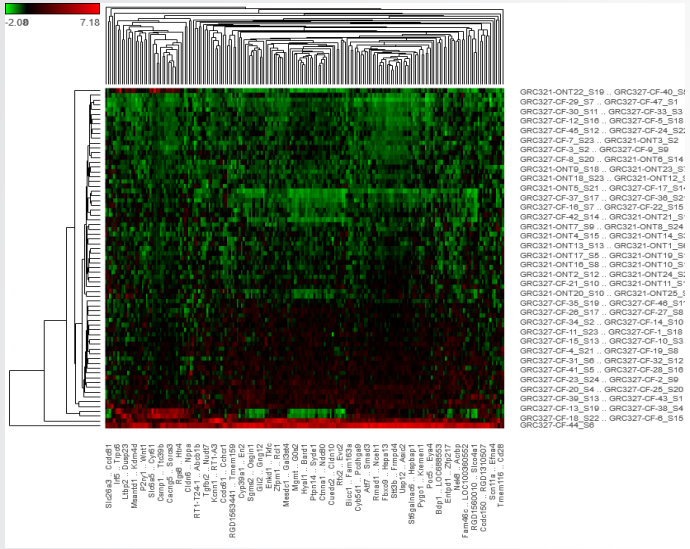






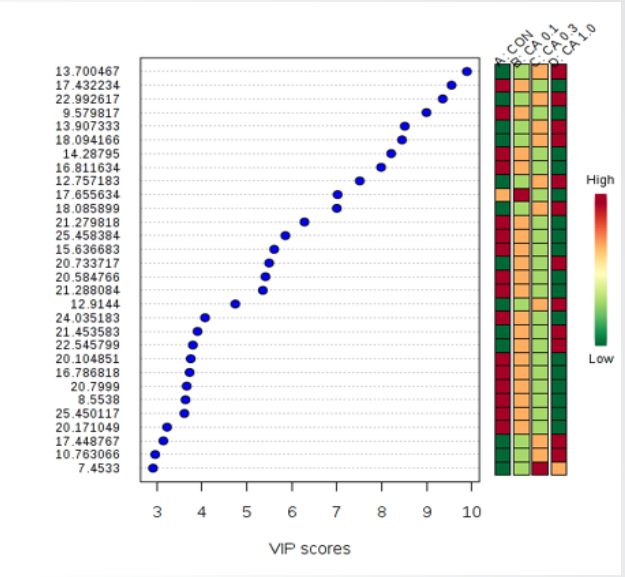


Critical concentration ("tipping point") determined



Six Chemical Proof of Concept

Domoic acid	Haloperidol
Cypermethrin	Deltamethrin
Cytosine Arabinoside	5-Fluorouracil

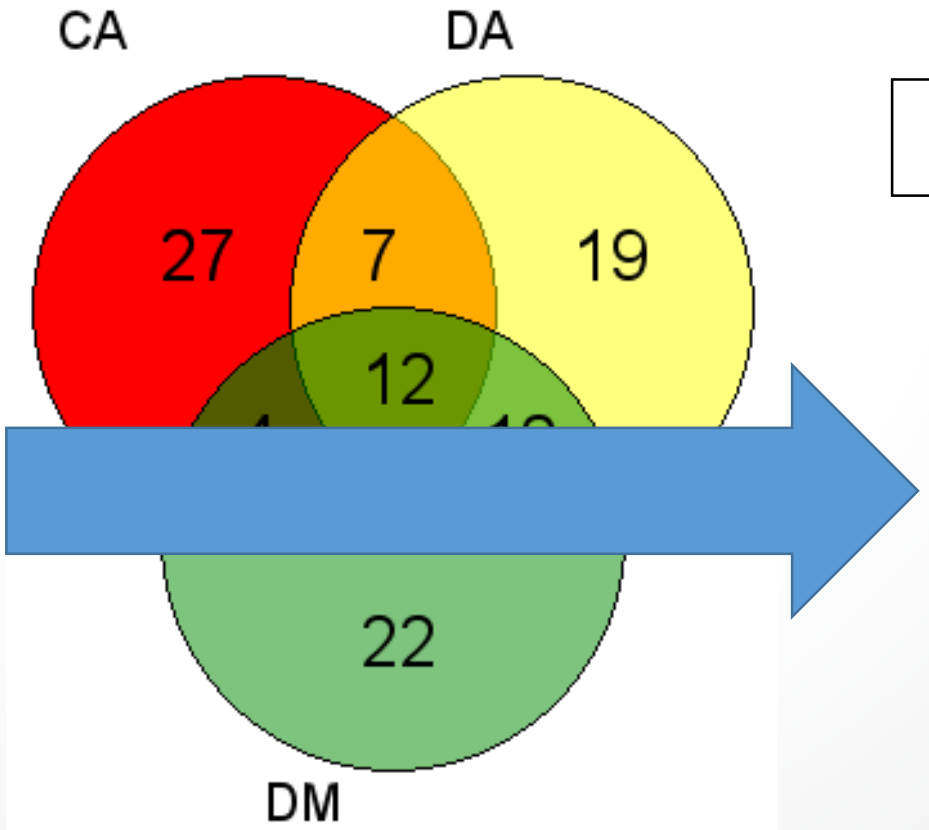


Canonical Pathway: Axonal Guidance

Transcriptomics

Found in all three gene lists

ACTR3	EFNA5
ADAM15	EPHA7
ADAMTS5	FZD2
BMP7	FZD5
BRCC3	FZD7
EFNA4	GLI2



Metabolomics

(How) are these genes altered in human neurodevelopmental abnormalities due to environmental or disease impacts?

High-throughput assays for DNT provide information for Adverse Outcome Pathway Development

Toxicant

Chemical Property Profile

→ **Molecular Initiating Event**

? (many postulated, few confirmed)

→ **Cellular Responses**

Key Events
proliferation
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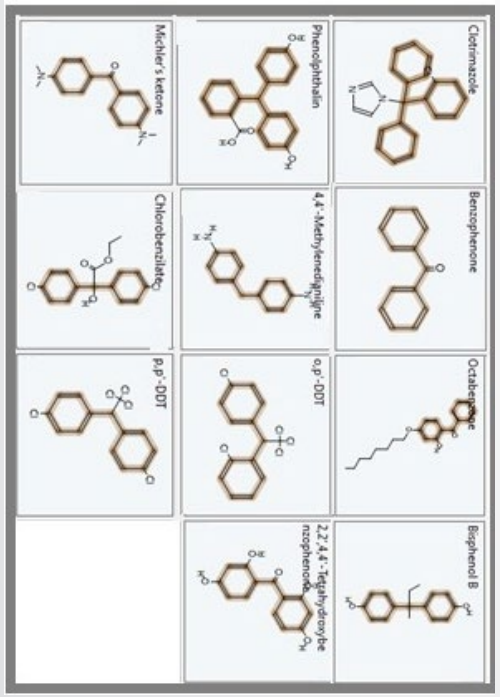

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neurotransmitter receptors
growth factor receptors
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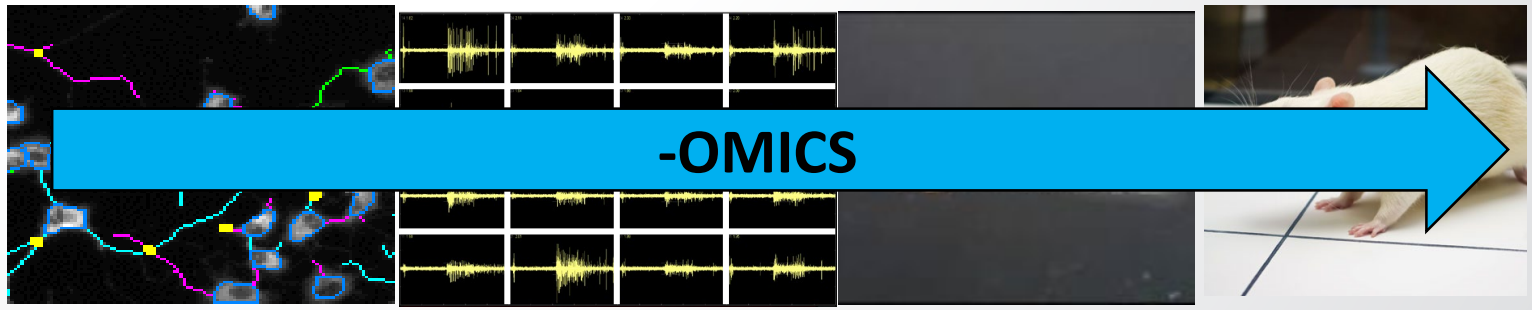
…

In Vitro Approaches

Behavior



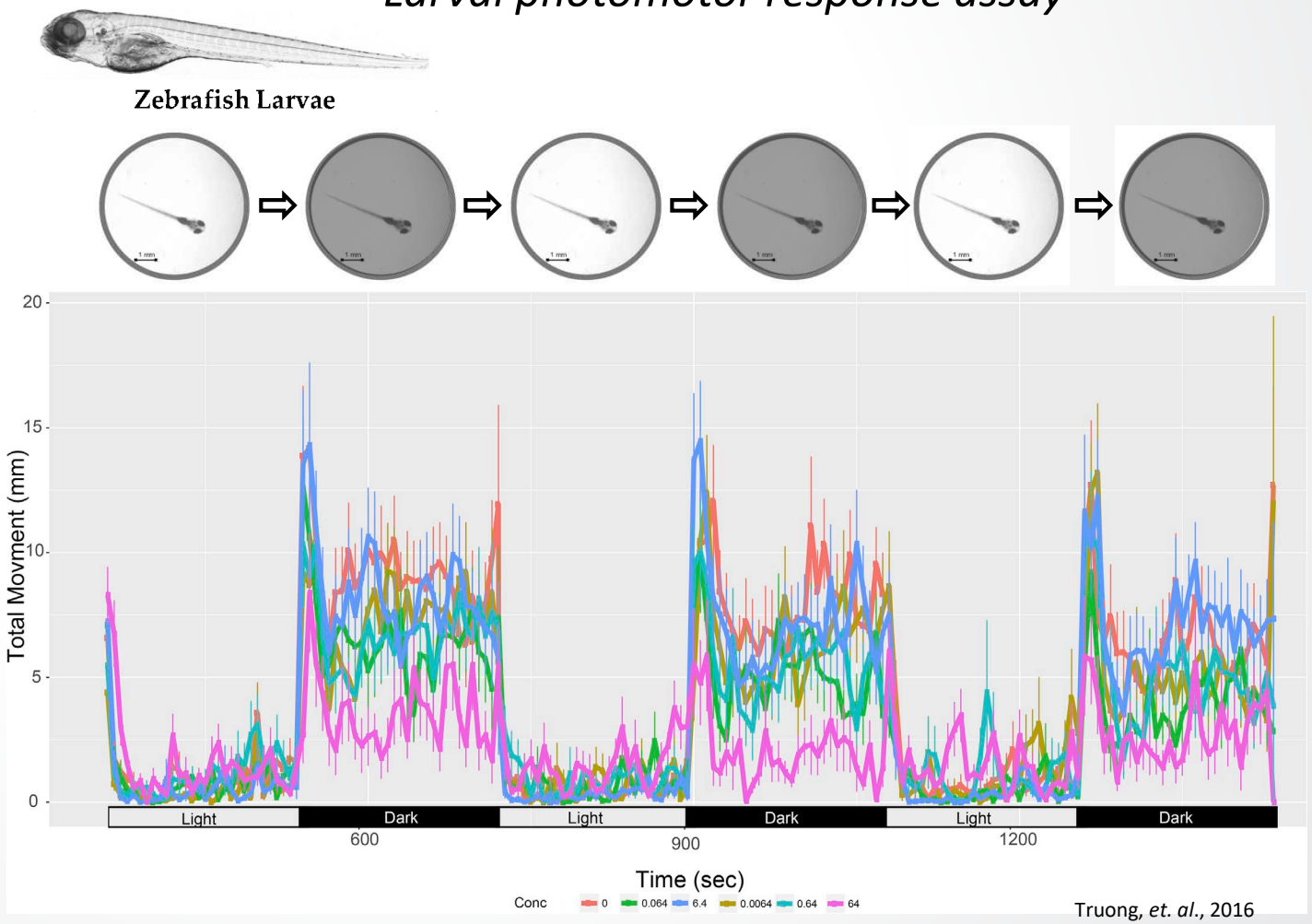
Future Directions

“Fish on a Chip”: Linking network formation and behavior

- Their ease of accessibility, genetic engineering, and behavioral screens make zebrafish useful models for many neurological diseases
- Compared to *in vitro* assays, *in vivo* behavioral assays more closely recapitulate human neurodevelopmental disorders
- Zebrafish have been established for the *in vivo* assessment of DNT
  - Larval photomotor response: an assay typically used to assess the zebrafish startle response
- Lack throughput and mechanistic information

Zebrafish Behavior:

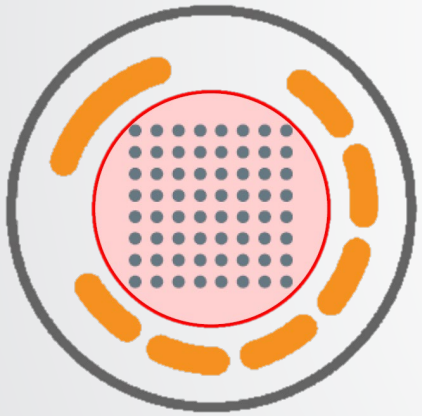
Larval photomotor response assay



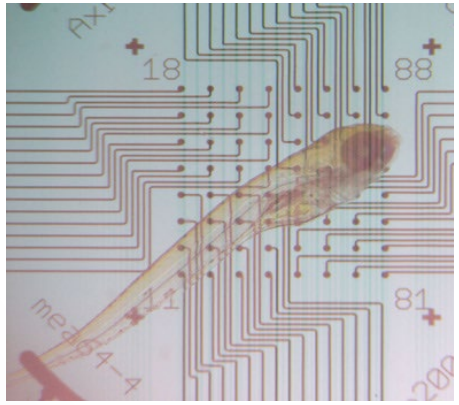
Truong, *et. al.*, 2016

Specific Aim 1:
Develop the zebrafish MEA protocol

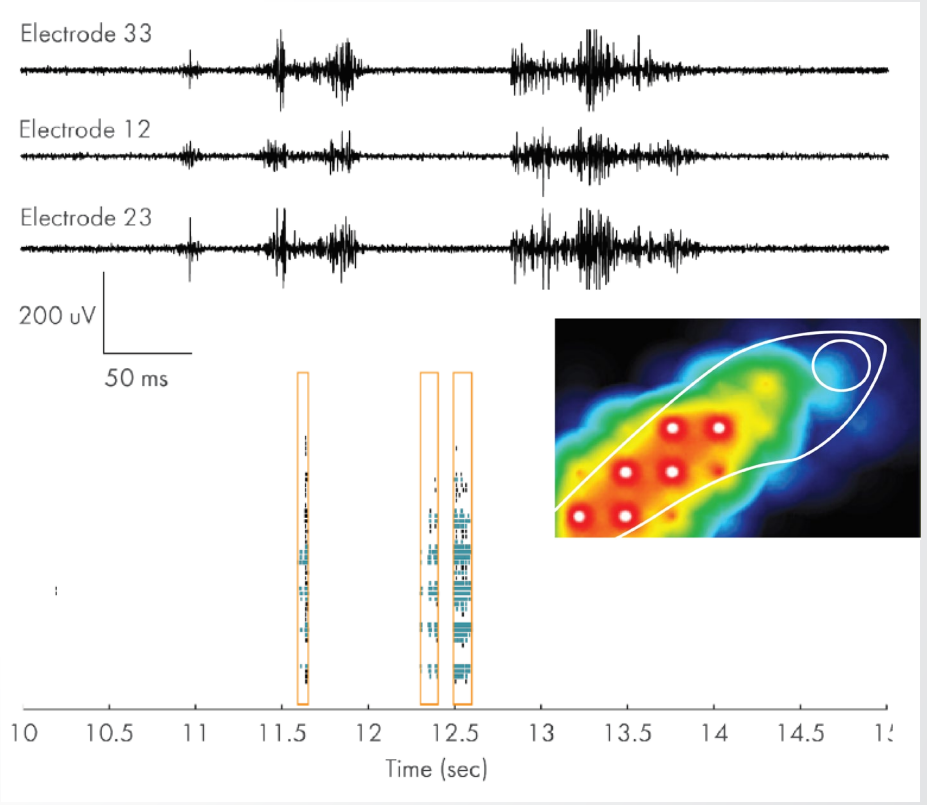
Well of an MEA plate



Place zebrafish in well and immobilize in agarose

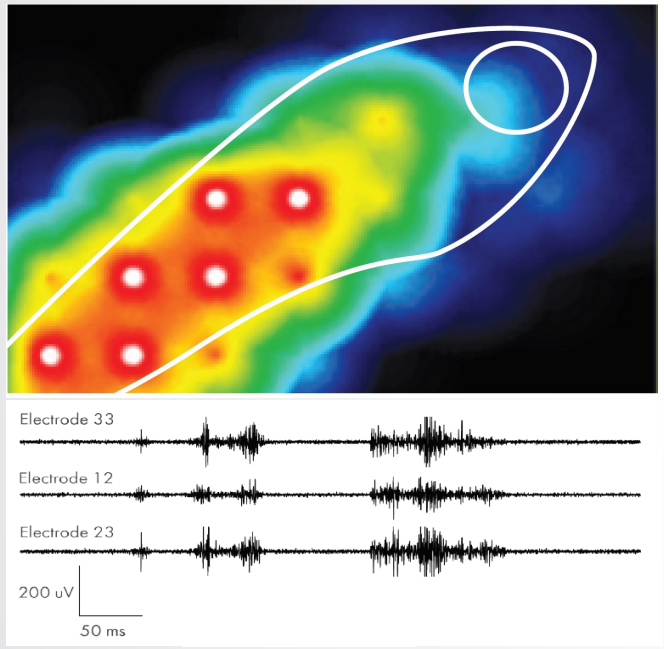


Record electrical activity from brain of zebrafish



Specific Aim 2: Develop a novel zebrafish larval photomotor response assay using MEA technology

Spontaneous electrical activity in brain of zebrafish larvae



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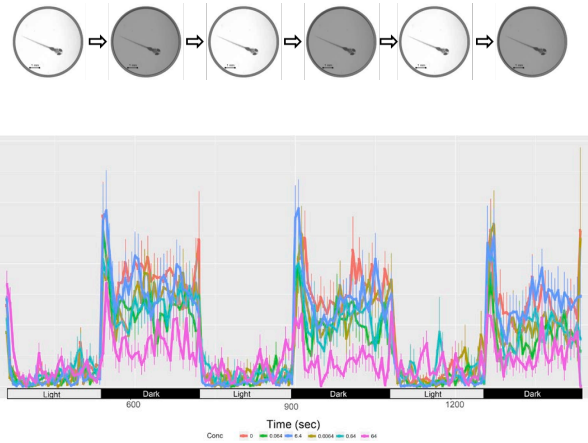
Zebrafish larval photomotor response assay



Truong, *et. al.*, 2016

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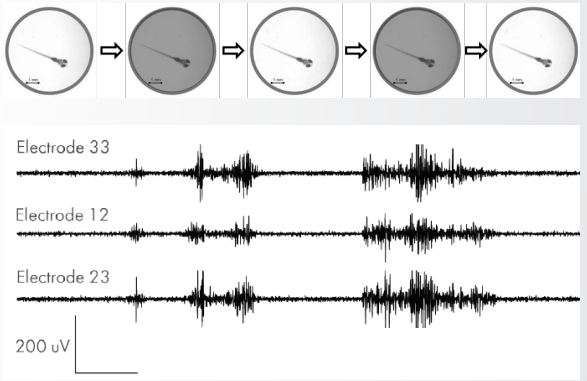
Modified larval photomotor response assay



Record electrical brain activity at baseline and during dark to light transition period

Implications

Implications:

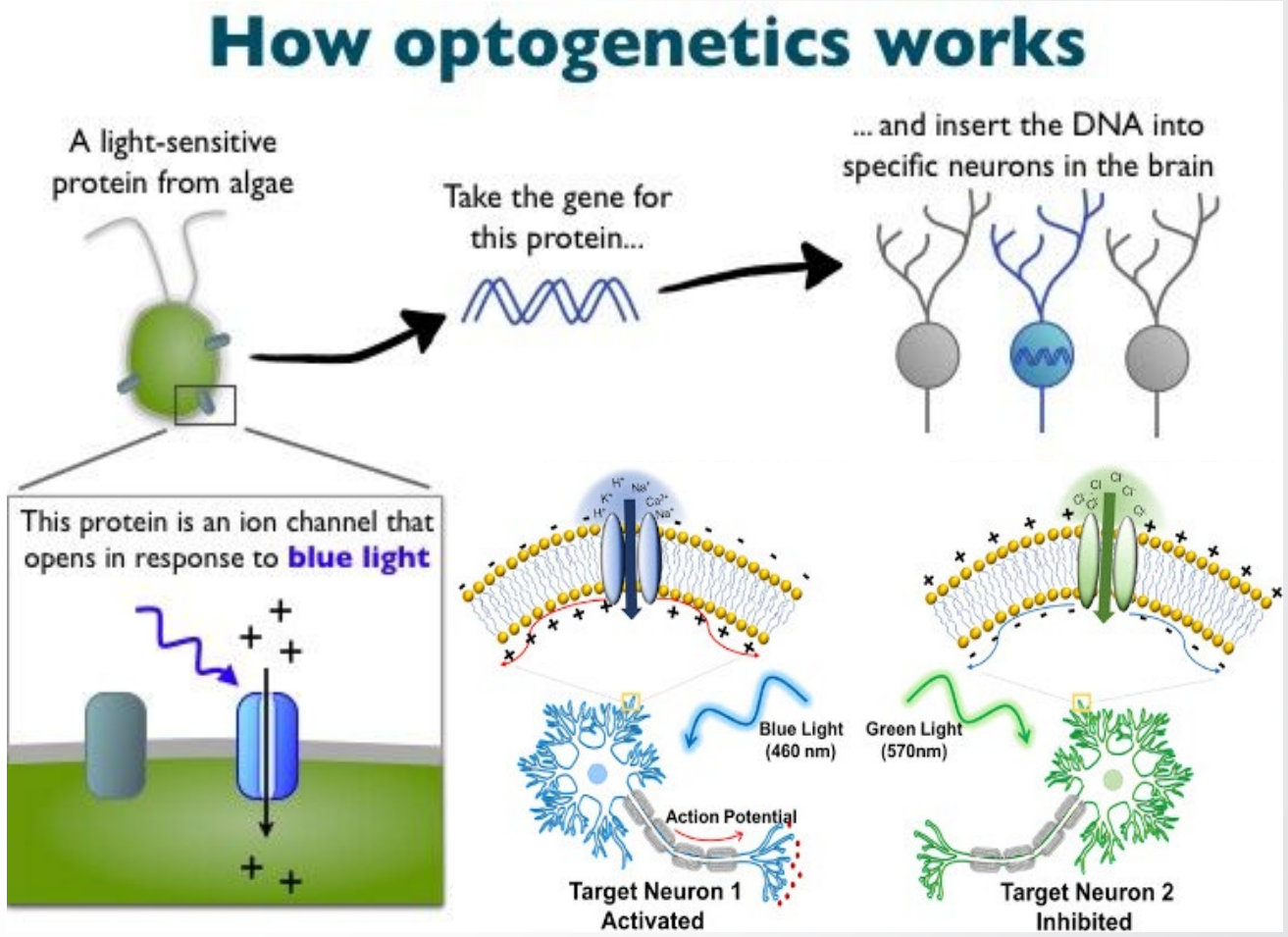
- This “Fish on a Chip” strategy will allow us to link behavior to neurophysiology by measuring zebrafish brain activity during the larval photomotor response assay
- Will also facilitate bridging the gap between *in vivo* and *in vitro* DNT assays and will improve the scientific basis for using *in vitro* data for decision-making

Future Directions:

- Will allow us to assess changes in brain activity and neural network development following chemical exposure
- Will pave the way for use of optogenetic approaches and other manipulations, which will facilitate mechanistic work to support the development of Adverse Outcome Pathways (AOPs) for DNT assessment.

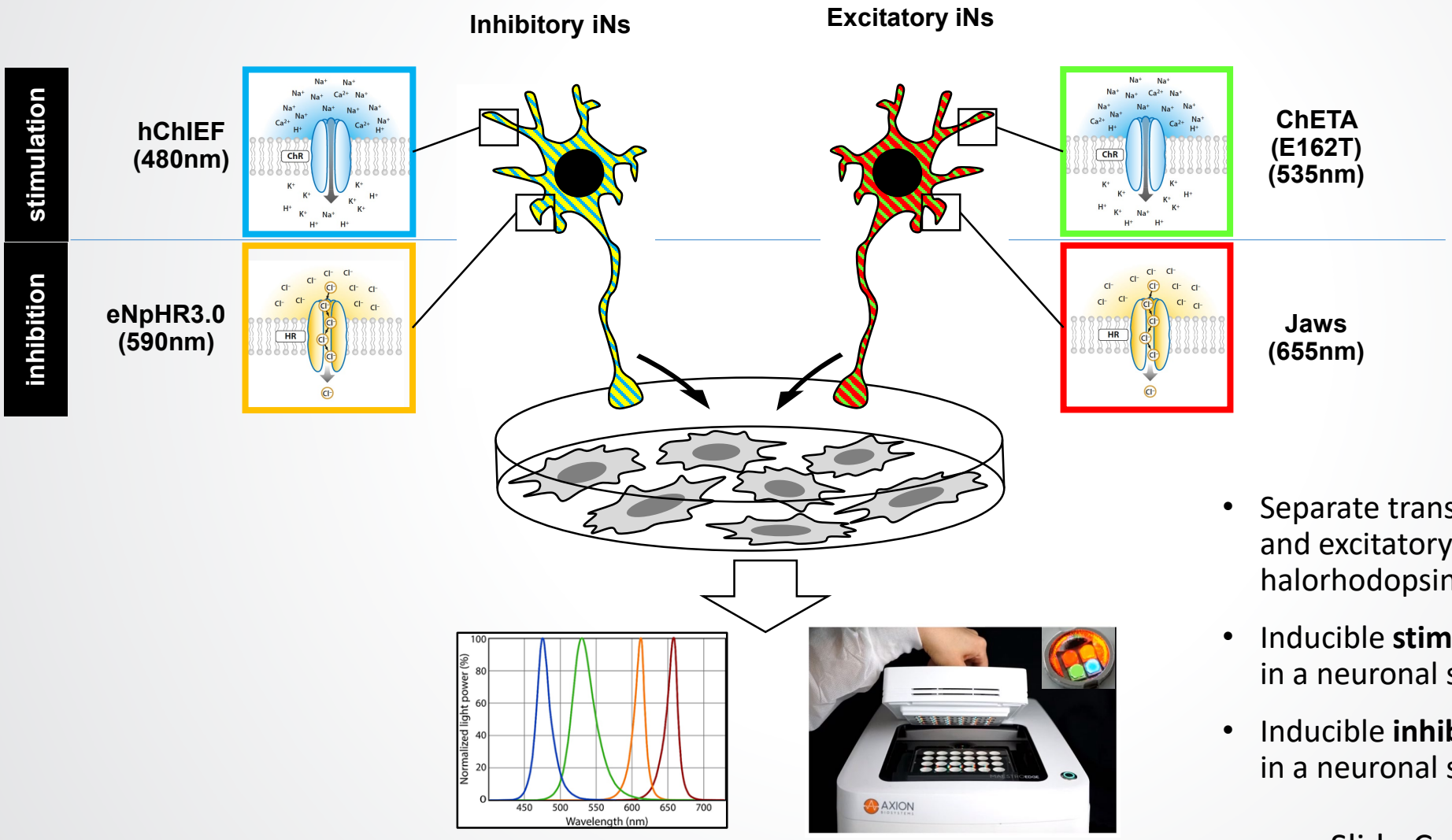
BrainGlo: Flipping the light switch on brain function

- Key processes such as plasticity, that may be associated with learning and memory, are not currently included in DNT NAMs.
- Optogenetics is a biological tool that uses light to control neurons that have been genetically modified to express light-sensitive ion channels
- Allows manipulation of activity of specific populations of neurons.



Ordaz, et. al., 2017

Specific Aim 1. Using optogenetic approaches, develop human neural network models in which we can specifically increase or decrease activity in excitatory or inhibitory neurons.

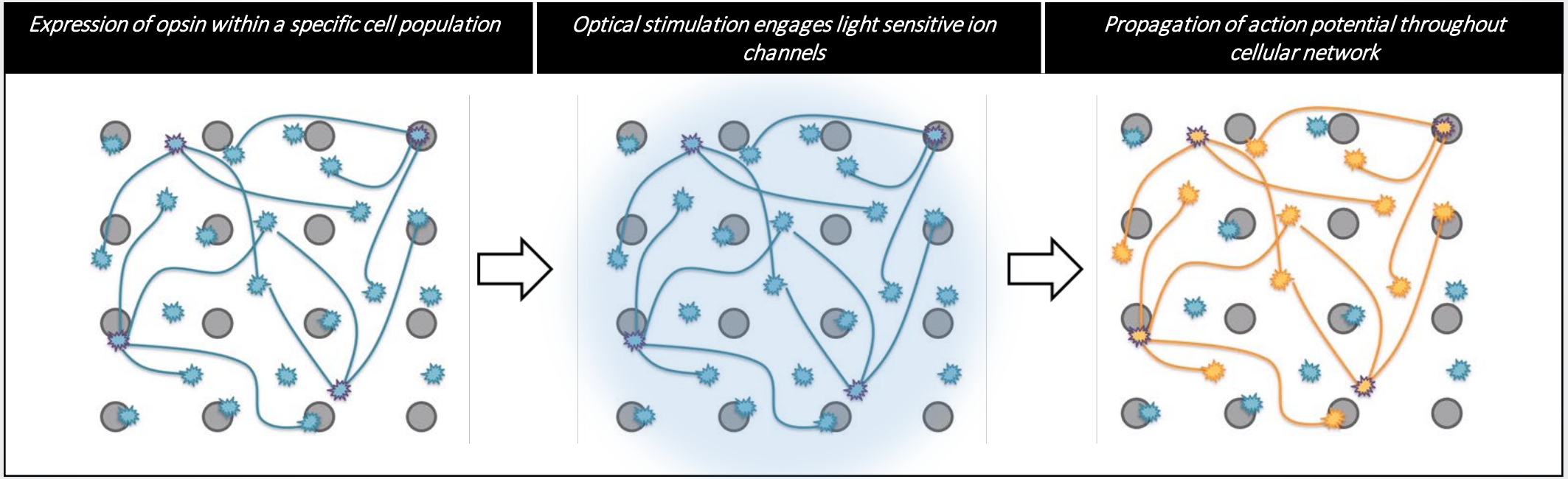


Lumos multi-well optical stimulator uses different wavelengths of light to activate the opsins

- Separate transduction of inhibitory iNs and excitatory iNs with rhodopsin and halorhodopsin variants
- Inducible **stimulation** of neuronal activity in a neuronal subpopulation
- Inducible **inhibition** of neuronal activity in a neuronal subpopulation

Slide Courtesy of NeuCyte

Specific Aim 1. Using optogenetic approaches, develop human neural network models in which we can specifically increase or decrease activity in excitatory or inhibitory neurons.

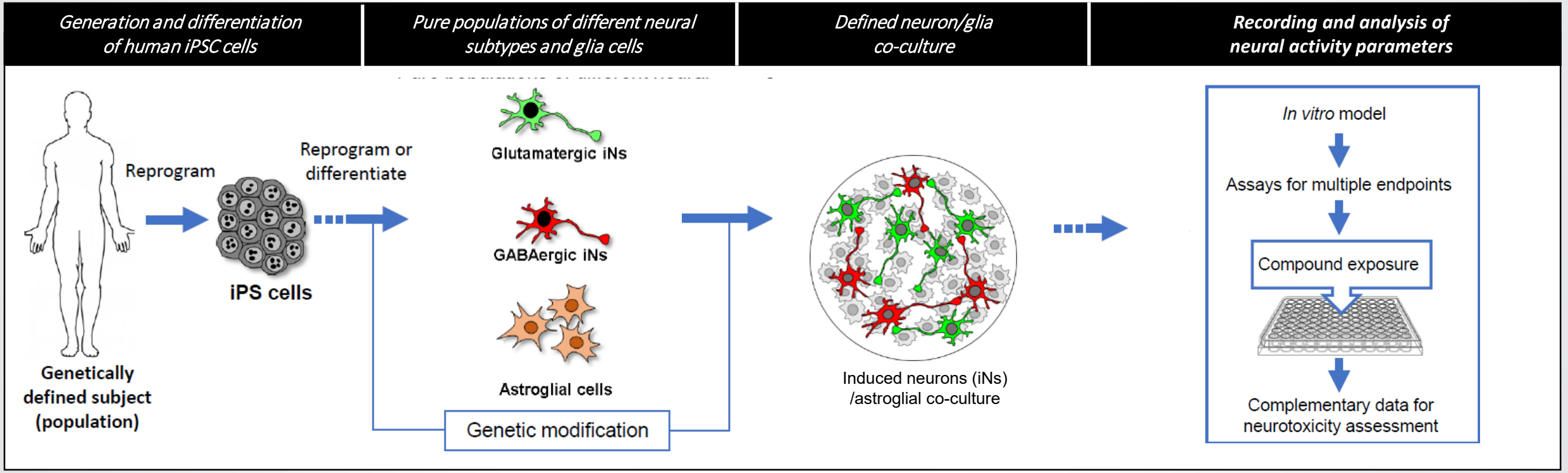


Optogenetic stimulation:

- Promoter-driven expression of opsins in certain cell types
- Well-wide illumination activates neurons expressing opsin
- Action potentials propagate through the neural network

Slide Courtesy of NeuCyte

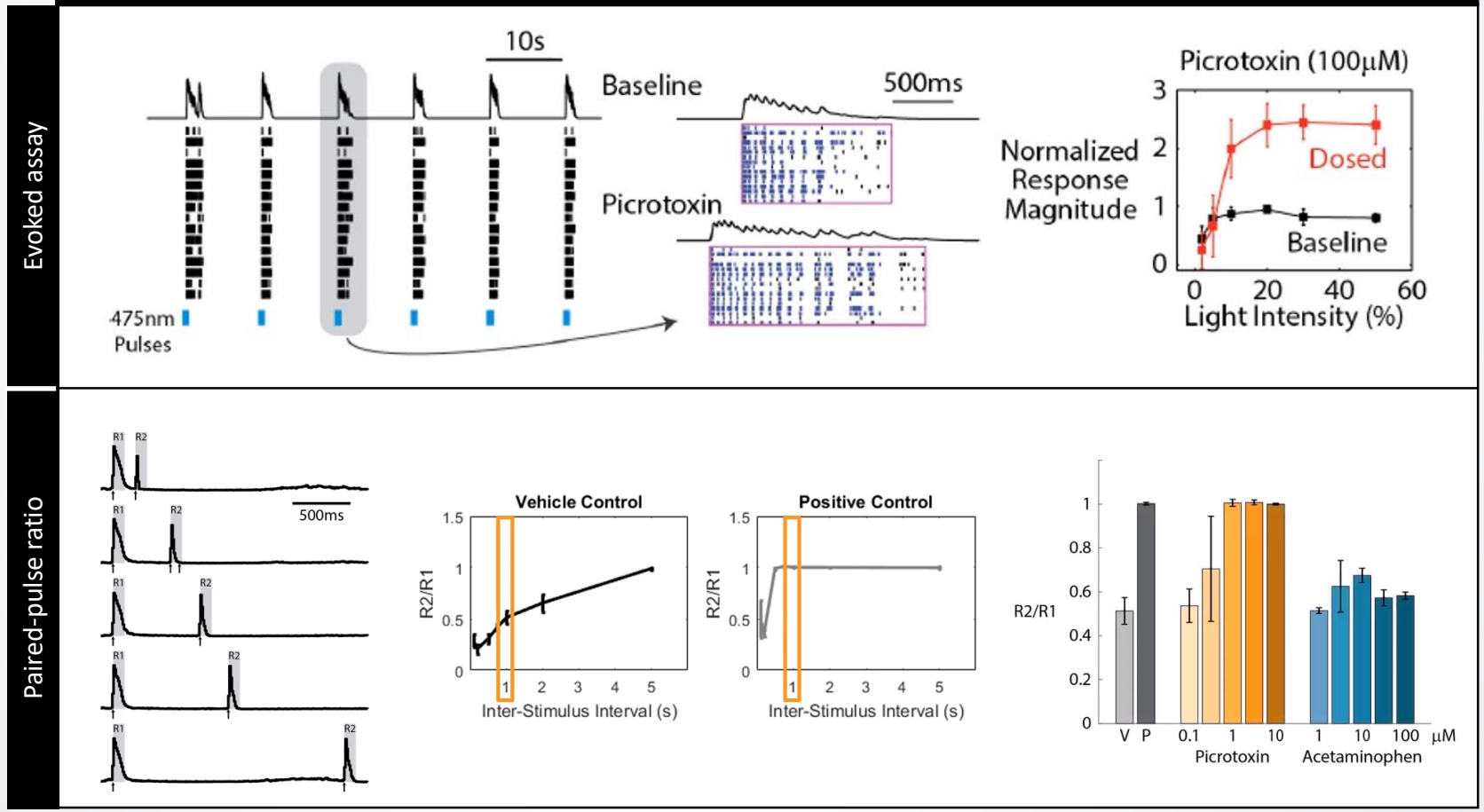
Specific Aim 2. Evaluate the effects of exposure to neurotoxicants on excitatory and inhibitory function in neural networks



Slide Courtesy of NeuCyte

Specific Aim 2. Evaluate the effects of exposure to neurotoxicants on excitatory and inhibitory function in neural networks

Using optogenetics to quantify changes in the balance of excitation and inhibition following chemical exposure



Slide Courtesy of NeuCyte

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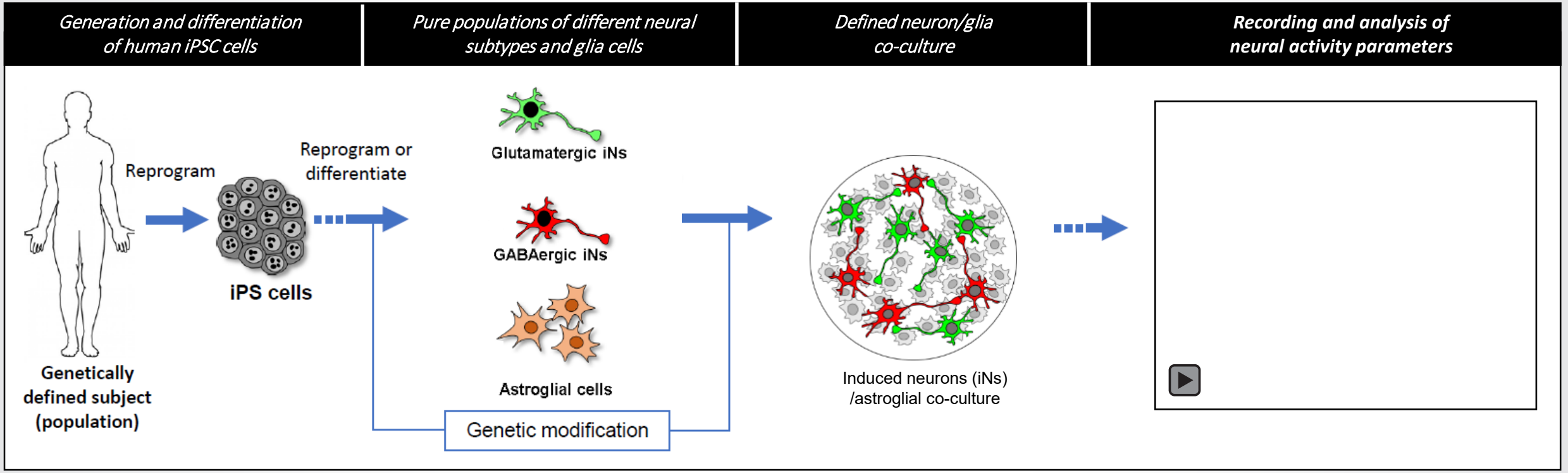
EPA Pathfinder Innovation Projects

- Network Formation Assay
- -Omics approaches (C Frank)
- Fish on a chip (M Martin)
- Optogenetics (M Martin)

Chemical			Concentrations	
Haloperidol		0.3	1.0	3.0
Domoic Acid		0.1	0.30	1.00
Deltamethrin		1	3.0	10.0
Cypermethrin		1	3.0	10.0
Cytosine Arabinoside		0.1	0.3	1.0
5-Fluorouracil		0.1	0.3	1.0
Chlorpyrifos		0.3	1.0	3.0
Chlorpyrifos Oxon		0.03	0.10	0.30
Lindane		0.3	1.0	3.0
Heptachlor Epoxide		0.3	1.0	3.0
Tebuconazole		0.3	1.0	3.0
Dieldrin		0.3	1.0	3.0
Cadmium Chloride		0.01	0.03	0.10
Lead Acetate		1.0	3.0	10.0
Permethrin		0.3	1.0	3.0
PBDE-47		0.3	1.0	3.0
TBT (bis-tri-n-butyltin oxide)		0.001	0.003	0.01
Triethyltin Bromide		0.003	0.01	0.03
Chlordiazepoxide		1	3	10
Emamectin		0.03	0.1	0.3
Flusilazole		0.03	0.1	0.3
Methylchloroisothiazoine		0.1	0.3	1.0
Paraquat		0.03	0.1	0.3
Sodium Arsenite		0.03	0.1	0.3

SynFire® human iPSC-derived induced neuron (iN)/glial co-culture system

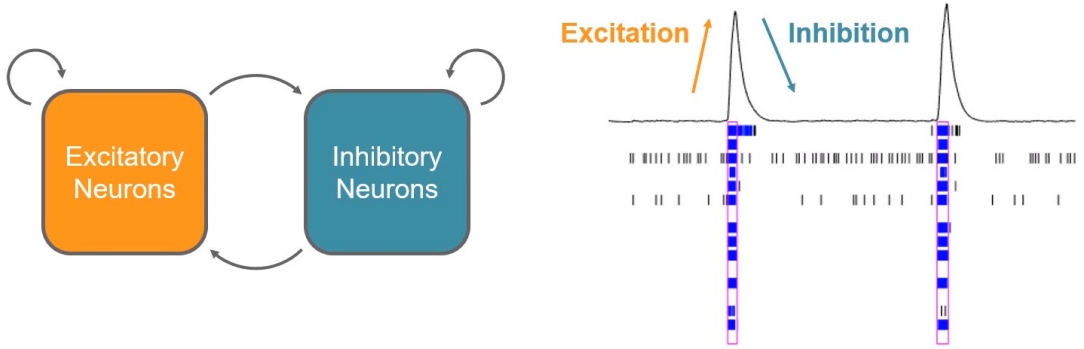
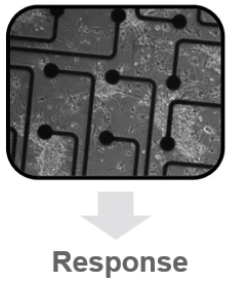
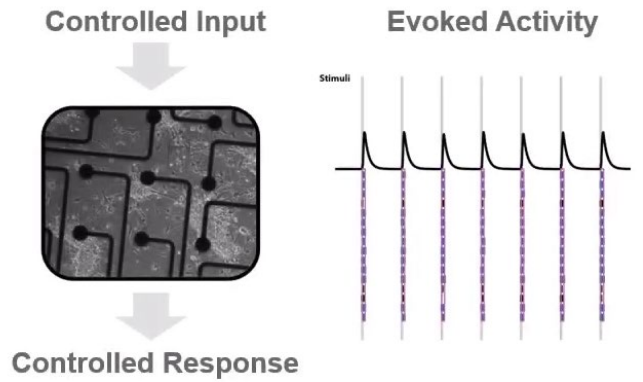


Synfire human iPSC-derived induced neurons (iNs) and glia

- Fast maturation and low variability
- Specified cell composition and reliable and robust readouts
- Cell-type specific modification for flexible assay design
- Rapid developing complex synchronized network activity

Slide Courtesy of NeuCyte

Specific Aim 1. Using optogenetic approaches, develop human neural network models in which we can activate specifically excitatory or inhibitory neurons.

Quantifying the balance of excitation and inhibition	*Spontaneous Assay*	*Evoked Assay*
		

Synfire® human iPSC-derived iNs and glia

- A network event begins with excitation and ends with inhibition, with the timing and intensity of the next event providing important information on the balance of excitation and inhibition
- **Evoked assays** - standardize the network activity across wells and conditions thereby reducing well to well variability. Allows new evoked assay endpoints.
- **Enhanced reliability** – controlled activity rates improve consistency across wells and can accelerate assay time scale

Slide Courtesy of NeuCyte