



HESI.

HESI Translation Biomarkers of Neurotoxicity (NeuTox) Committee

2020 Mid-Year Update

About HESI



INTERNATIONAL NON-
PROFIT BUILDING
SCIENCE FOR A SAFER,
MORE SUSTAINABLE
WORLD



31 YEARS OF OUTCOME
DRIVEN SCIENCE



CROSS-SECTOR
COLLABORATIONS
TOWARDS ENHANCING
THE QUALITY OF GLOBAL
HUMAN HEALTH SAFETY
ASSESSMENT

The HESI Model: Bridging Research to Application

30
THIRTY YEARS
HESI

*Science for a Safer,
More Sustainable World*

IMPROVED

SAFETY AND INNOVATION FOR HUMAN AND ENVIRONMENTAL HEALTH



Academic, Clinical,
& Research Scientists
& Organizations



Industry
Research &
Development



NGOs, Patient
Advocacy Groups,
& Foundations



Government
Research &
Regulation





Committee Mission

The committee's mission is to identify (i) biomarkers for improving the prediction of neurotoxicity and (ii) seizurogenic compounds using microelectrode array (MEA).

Co-Chairs and Staff

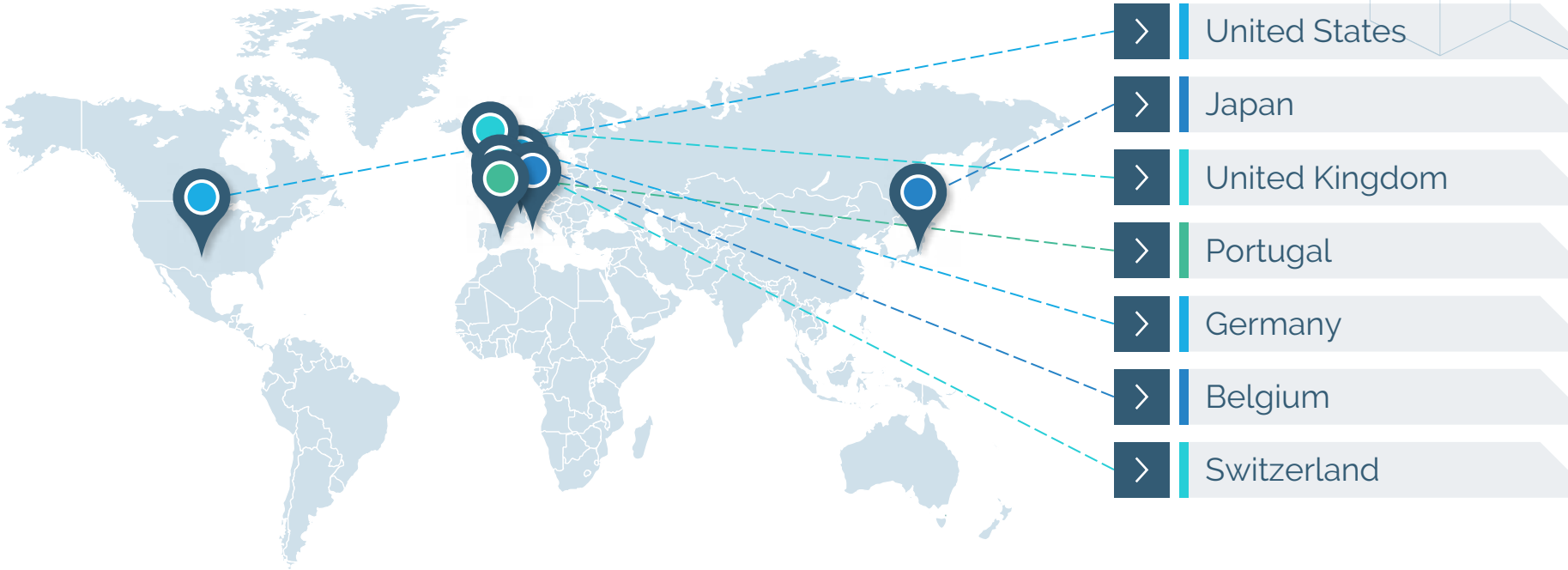


Dr. William Slikker, US Food & Drug Administration
Dr. Ruth Roberts, ApconiX



Ms. Jennifer Pierson
HESI Senior Scientific Program Manager

Geographic Representation



2020 Participating Organizations

Government/Regulatory Agencies

- ▶ National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs, UK)
- ▶ National Institute of Health Sciences (Japan)
- ▶ National Institutes of Health
- ▶ Swiss Center for Applied Toxicology
- ▶ US Army
- ▶ US Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health
- ▶ US Environmental Protection Agency
- ▶ US Food and Drug Administration

Academic/Research Institutions

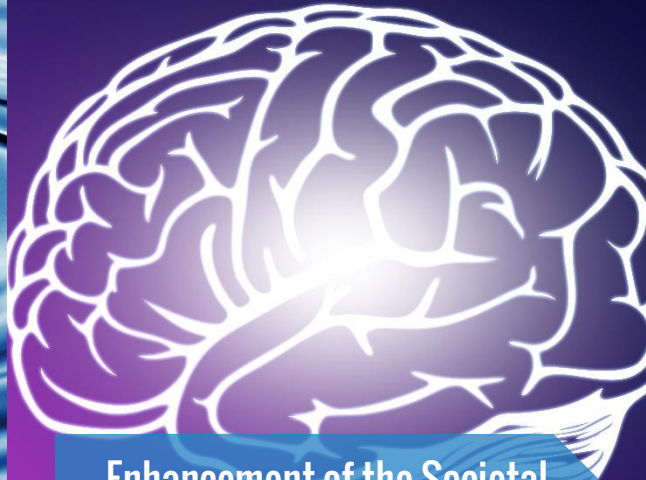
- ▶ Albert Einstein College of Medicine
- ▶ Colorado State University
- ▶ Duke University
- ▶ Gunma University
- ▶ Natural and Medicines Institute, University of Tubingen
- ▶ Newcastle University
- ▶ Purdue University
- ▶ Tohoku Institute of Technology
- ▶ Tokyo, Graduate School of Pharmaceutical Sciences
- ▶ University of Lisbon
- ▶ Utrecht University
- ▶ Virginia Tech

Industry

- ▶ Apconix
- ▶ Axosim
- ▶ Cellular Dynamics International, A Fujifilm Company
- ▶ Charles River Laboratories
- ▶ Cyprotex
- ▶ Elixirgen Scientific
- ▶ GlaxoSmithKline
- ▶ Janssen Pharmaceuticals
- ▶ Neucyte
- ▶ Pfizer Inc.
- ▶ Stemonix
- ▶ Sumitomo Dainippon Pharma
- ▶ Takeda Pharmaceutical Company Limited



Catalysis of New Science

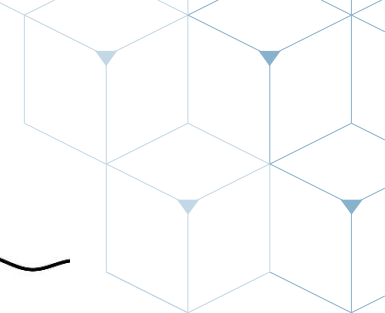
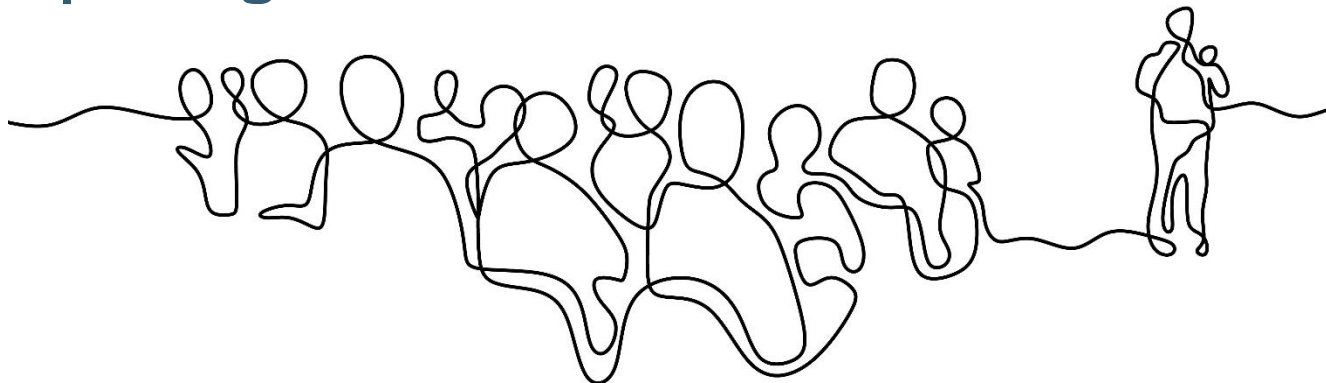


Enhancement of the Societal Knowledge Base on Human Biological Processes of Relevance for Protecting Human Health



Increasing the Audiences for Collaborative Safety Science

Recent & Upcoming Outreach



March 2019

SOT 2019
Annual Meeting
(Baltimore, MD)

July 2019

FDA Workshop
on Biomarkers
of Neurotoxicity
(FDA White Oak)

Sept 2019

Safety
Pharmacology
Society Annual
Meeting
(Barcelona,
Spain)

August 2020

National Academy
of Sciences
Workshop on
Predicting Human
Health Effects
from
Environmental
Exposures

2021

EuroTox 2021
(Copenhagen,
Denmark)

Recent Publications



He Z, Panos J, Raymick J, Konak T, Cui L, Miller DB, O'Callaghan JP, Liachenko S, Paule MG, Imam SZ (2019) A method for sampling rat cerebrospinal fluid with minimal blood contamination: a critical tool for biomarker studies. In Aschner M and Costa L (eds) *Cell Culture Techniques*. New York, NY: Humana. pp. 233–243. doi: 10.1007/978-1-4939-9228-7_12.



Roberts R, Authier S, Mellon D, Morton M, Suzuki I, Tjalkens RB, Valentin, J, Pierson JB. Can we panelize seizure? *Submitted Toxicological Sciences*



Imam et al. Study to investigate circulating biomarkers that predict central & peripheral neurotoxicity resulting from exposure to trimethyltin (TMT). *Final draft in progress.*

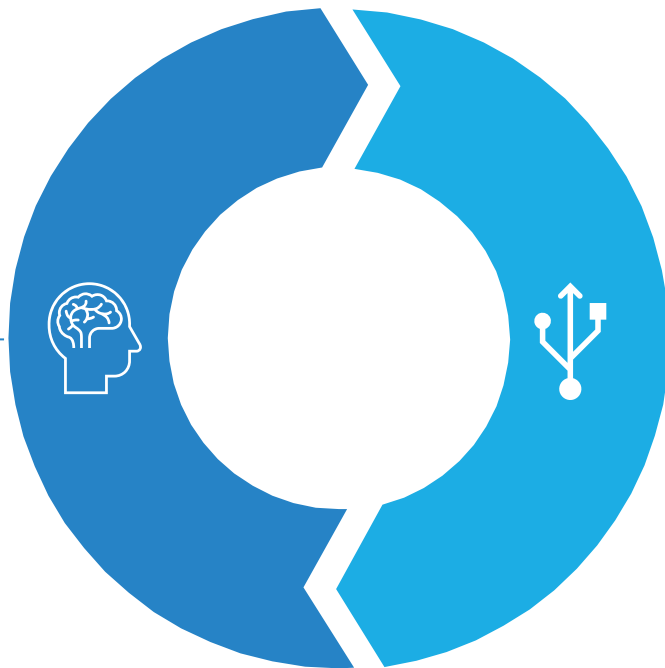


Shafer *et al.*, Detection of seizurogenic compounds using neural networks grown on microelectrode arrays; a multi-laboratory, multi-model assessment *Draft in progress*

Working Groups

Translational Biomarkers of Neurotoxicity

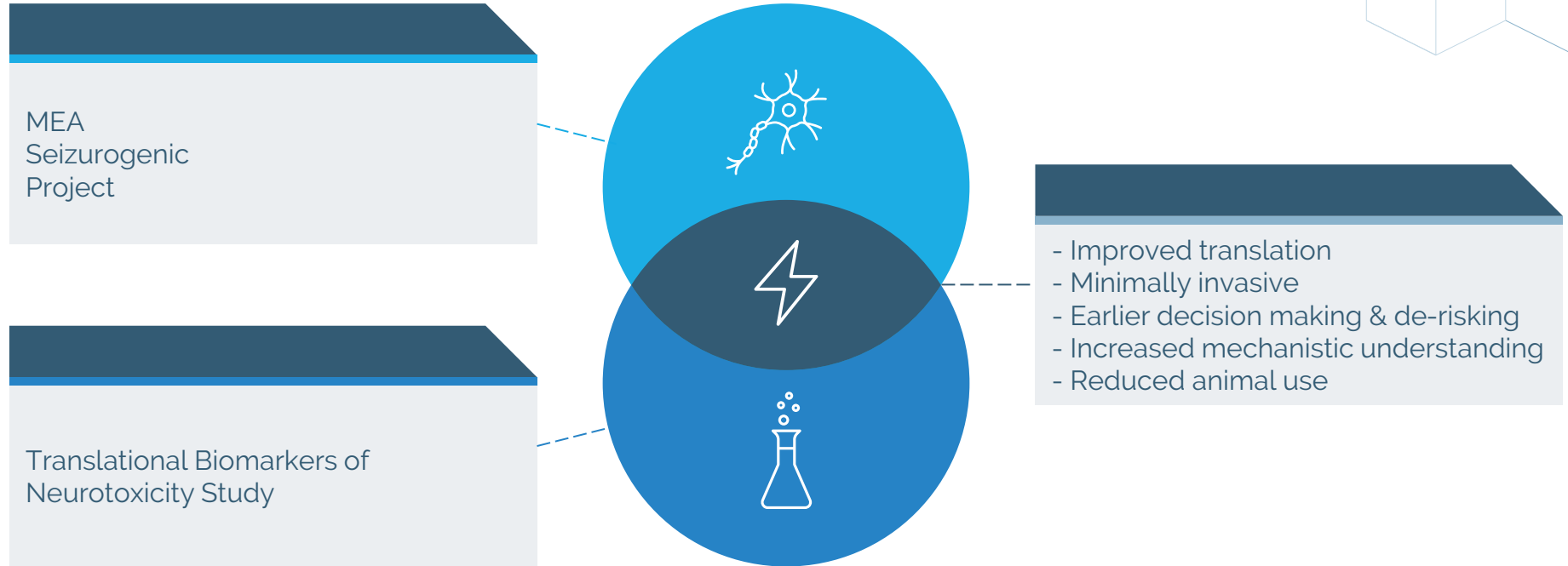
Working toward identifying biomarkers of improving the prediction of neurotoxicity and identifying correlates in behavioral, imaging, and neuropathological endpoints.



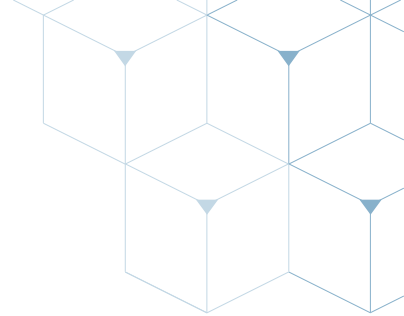
Microelectrode Array Subteam

Working toward characterizing the predictivity of seizurogenic activity using MEA technology

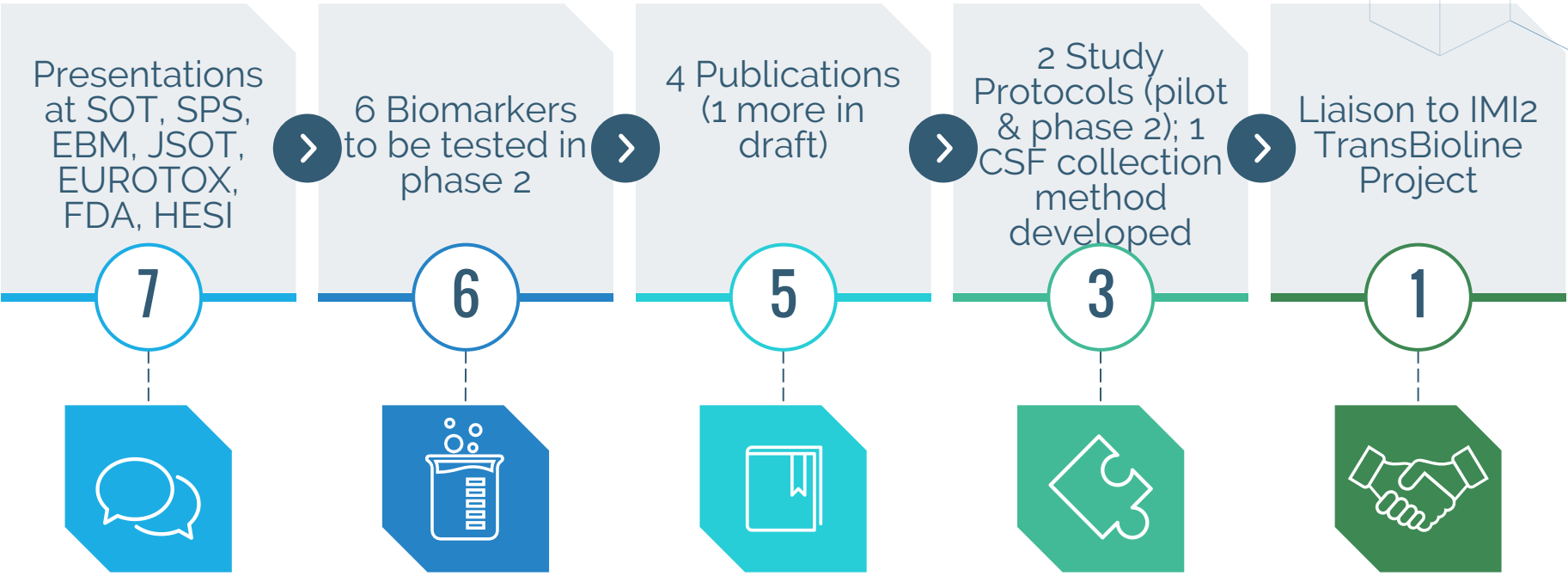
Project Synergy



Biomarker Pilot Study



Biomarkers Study - Accomplishments to Date



Biomarker Pilot Study Protocol

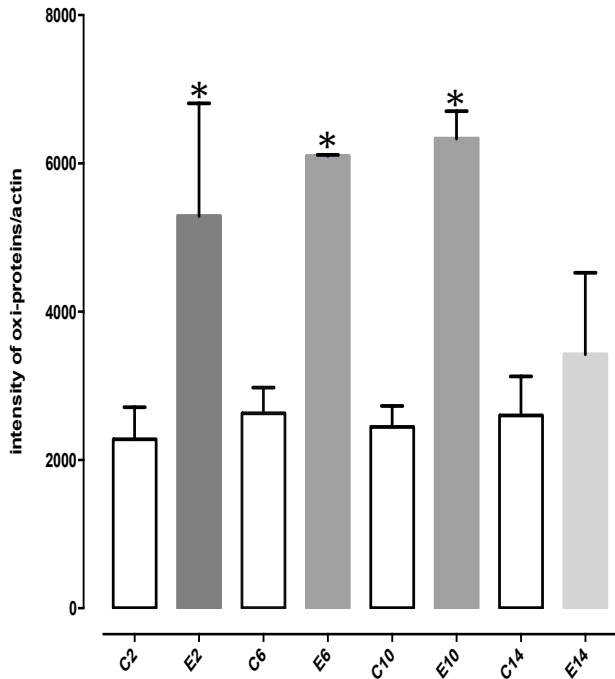
Objective: Identify circulating biomarkers that predict central and peripheral neurotoxicity by correlating them with behavioral, imaging, morphometric and neuropathological endpoints.

- ▶ Adult male, Sprague Dawley rats exposed via IP TMT at 8mg/kg
- ▶ Behavioral observations and samples collected at 2, 6, 10, 14 and 21 days post exposure
- ▶ Collected Biological Fluids – CSF, Plasma, Serum, Urine
- ▶ Collected Tissue Samples – Brain, Liver, Thymus, Adrenal, Kidney, Spinal Cord, Sciatic
- ▶ Measured Endpoints: Behavior, MRI, Proteomics, Histopath, Oxidative Biology, Bioplex Assays, GFAP, Metabolomics, Lipidomics, miRNAs

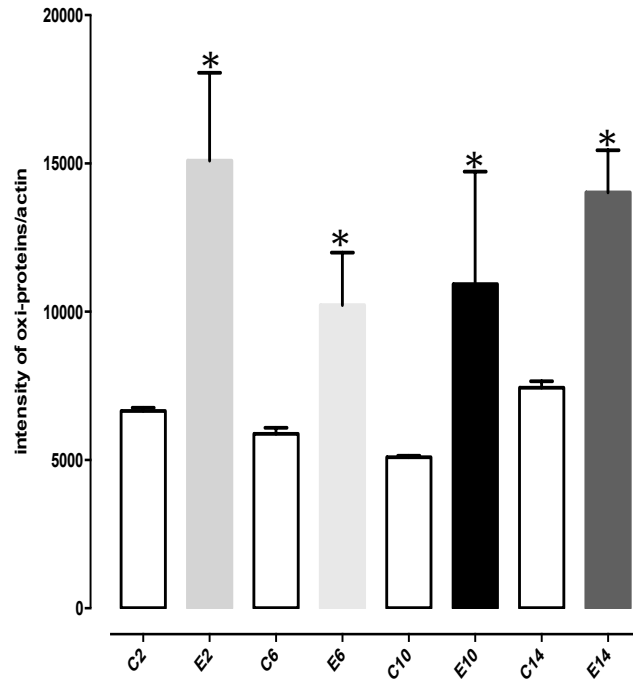
Stress → Damage: Neurotoxicity Markers

Oxidation, Inflammation, Metabolome & Lipidome

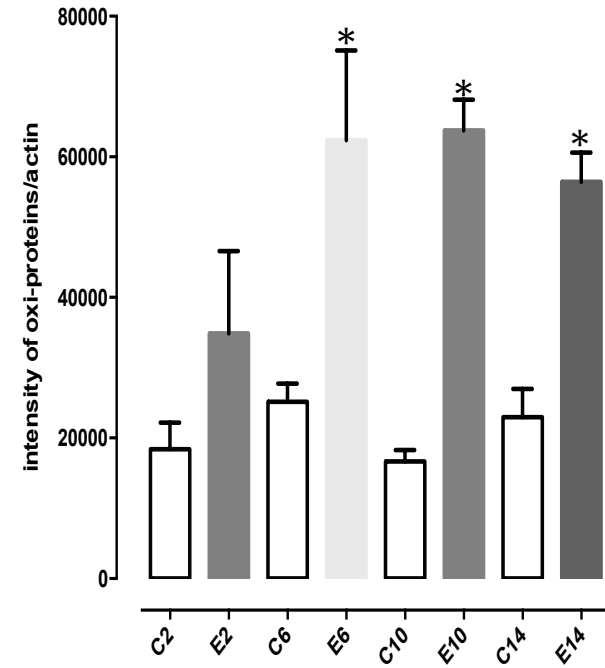
PLASMA - Oxidative Stress



SERUM - Oxidative Stress

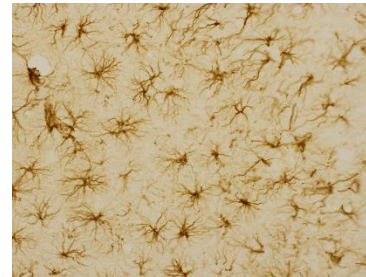
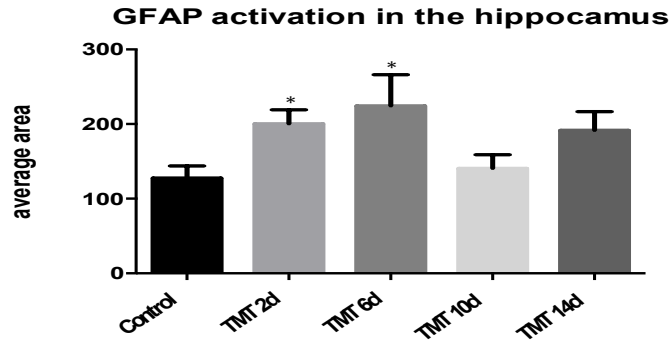
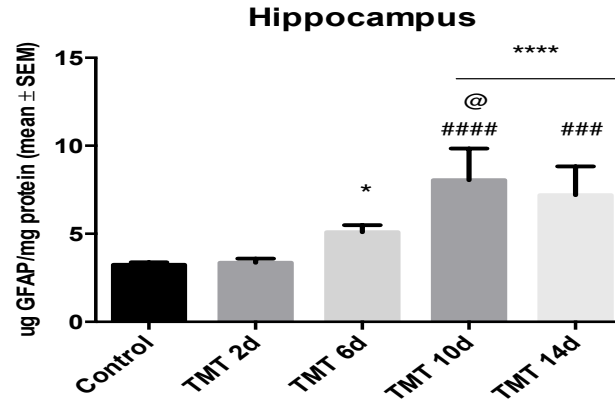
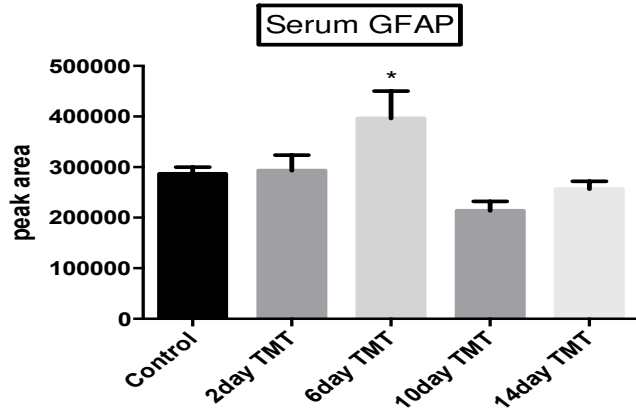


Hippocampus - Oxidative Stress



Stress → Damage: Neurotoxicity Markers

Oxidation, Inflammation, Metabolome & Lipidome



Control



TMT

Biomarker Pilot Study Results



Significant increase in the levels of oxidized protein
– Correlation seen between fluids and brain



Cytokines, TGF, UCHL-1 and Acylcarnitine –
Correlation seen between fluids and brain

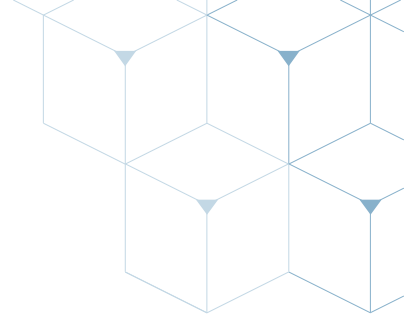


GFAP contents show a time dependent increase
– Correlation seen between fluids and brain



Translation to Clinical:
UCHL-1 and GFAP recently approved a clinical biomarkers of traumatic brain injury

Next Steps - Biomarker Pilot Study



One final publications with pilot study results in draft. Additional abstract submissions for future meetings TBD.



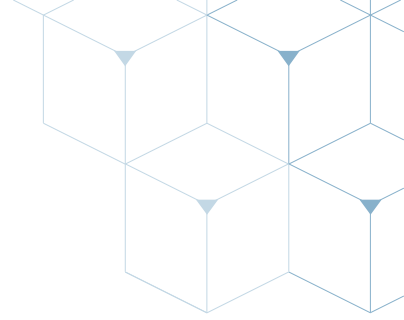
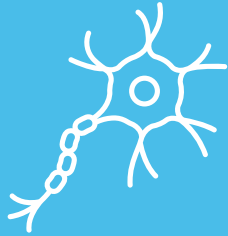
Phase 2 study planned. Protocol pending at FDA NCTR.

Biomarker Phase 2 Study Protocol

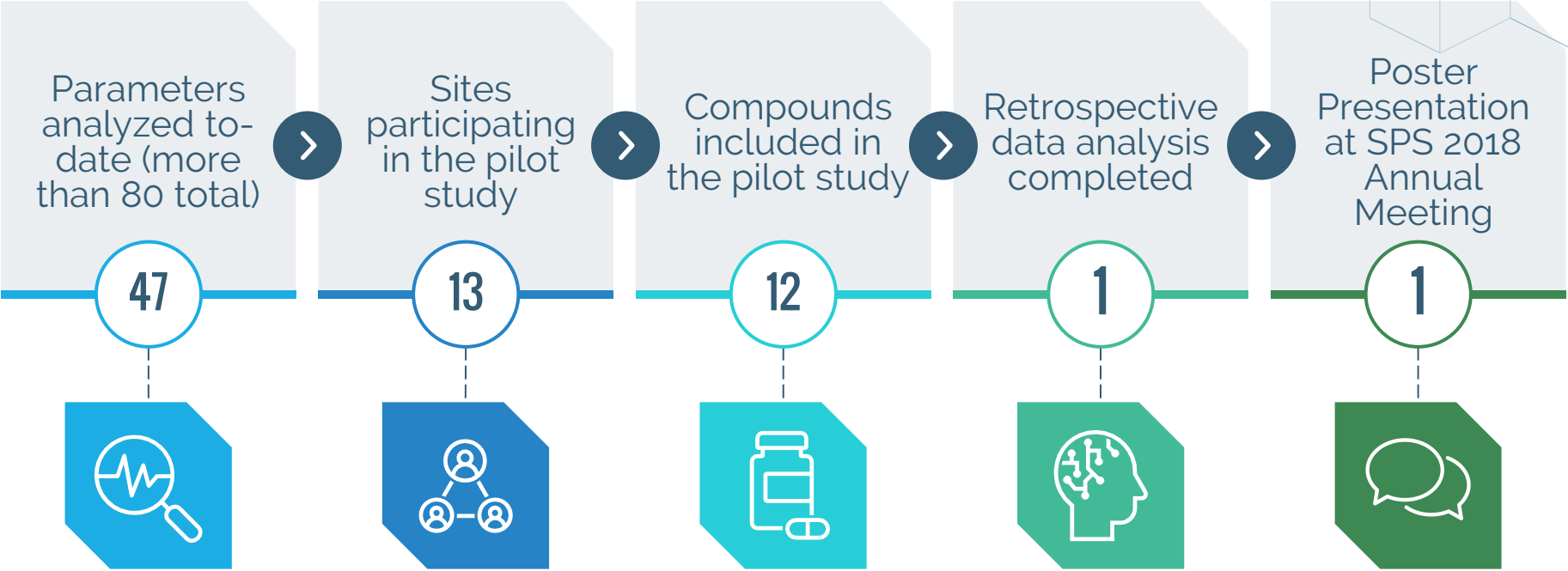
Objective: Validate the CNS-correlated fluidic biomarkers from phase 1 study.

- ▶ Adult male, Sprague Dawley rats exposed to (1) rotenone and (2) cuprizone
- ▶ Initial dose finding study planned as phase 1
- ▶ Phase 2 to include doses from phase 2 for (1) rotenone, (2) cuprizone and (3) acetaminophen (negative control)
- ▶ Behavioral observations and samples collected at 72 hrs, 1wk, 2wk, 3wk and 4wk post exposure
- ▶ Collected Biological Fluids – CSF, Plasma, Serum, Urine
- ▶ Collected Tissue Samples – Brain, Liver, Thymus, Adrenal, Kidney, Spinal Cord, Sciatic
- ▶ Measured Endpoints: MRI, Histopath, GFAP, UCHL-1, Neurofilament light, Metabolomics, Protein/Cytokines, Lipidomics

MEA Pilot Study



MEA Pilot Study - Accomplishments to Date



MEA Pilot Study Goals

- ▶ Quantify reliability of network phenotypes across wells, plates, and sites for each cell-platform combination.
- ▶ Identify assay endpoints to quantify network phenotypes and respond in a dose-dependent manner to neuroactive compounds, relative to vehicle controls, for each cell-platform combination.
- ▶ Assess the degree to which significant assay endpoints are correlated across seizurogenic compounds in the test set for each cell-platform combination.
- ▶ Assess the degree to which significant assay endpoints are correlated across cell-platform combinations for a given compound.

MEA Pilot Study Compounds

Compounds	Concentration Range (μM)
Pentylentetrazole	100-3000
Picrotoxin (PTX)	0.1-50
Strychnine	1-50
Pilocarpine	1-100
Chlorpromazine	0.1-10
Amoxapine	0.1-10
Isoniazid	25-500
Phenytoin	1-50
Linopirdine	1-60
4-Aminopiridine	0.1-100
Amoxicillin	0.1-100
Acetaminophen	0.1-100

MEA Pilot Study Participating Sites

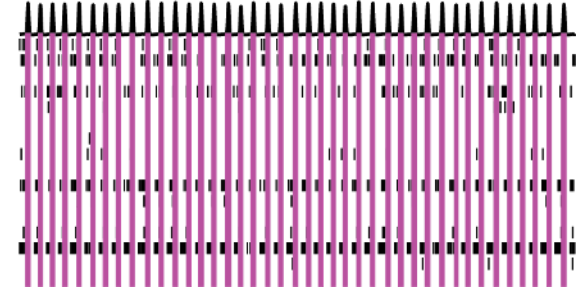
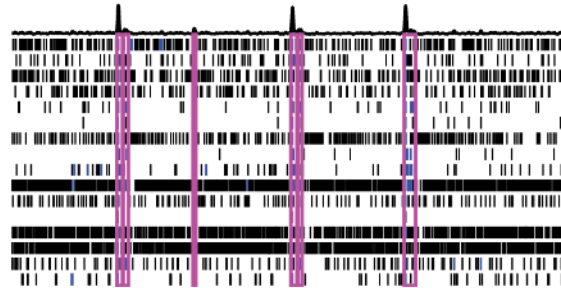
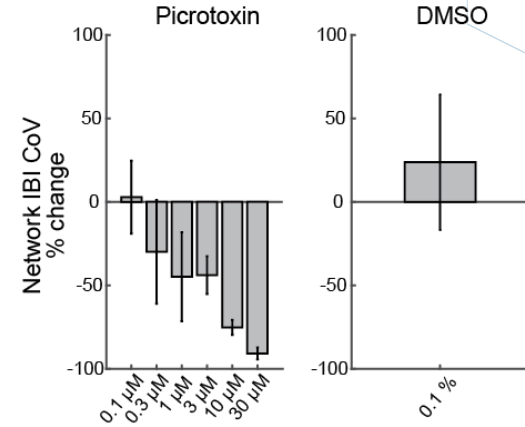
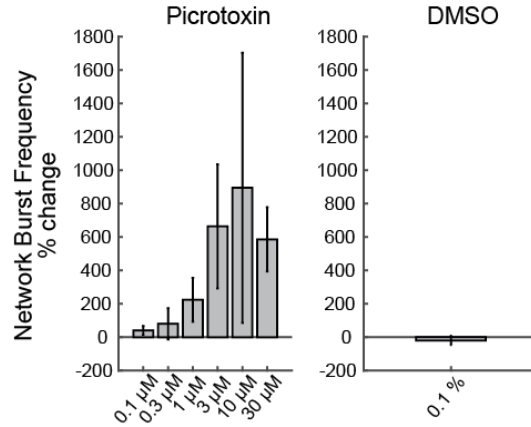


Site	Platform	Cell Type 1	Cell Type 2
Axion	Axion Maestro	Rat cortical	
BMS	Axion Maestro	CDI GlutaNeuron+Astrocyte	
Cyprotex	Axion Maestro	CDI GlutaNeuron+Astrocyte	Rat cortical
Eisai	AlphaMed, Axion Maestro	Rat cortical	
EPA	Axion Maestro	Rat cortical	
GSK	Axion Maestro APEX	Rat cortical	
JNJ	Axion Maestro	Rat cortical	iPSC (CNS4U)
Ncardia	Axion Maestro	iPSC (CNS4U) + Astrocyte	
NeuCyte	Axion Maestro APEX	iPSC (SynFire)	
NIHS Japan	AlphaMed	Rat cortical	
Tohoku	AlphaMed	Rat hippocampal	iPSC (AXOL)
CDI	Axion Maestro	CDI GlutaNeuron+Astrocyte	
FDA	Axion Maestro	iPS-derived GABA neurons co-cultured with iPS-derived astrocytes.	

MEA Pilot Study Initial Results

Rat Cortical Neurons

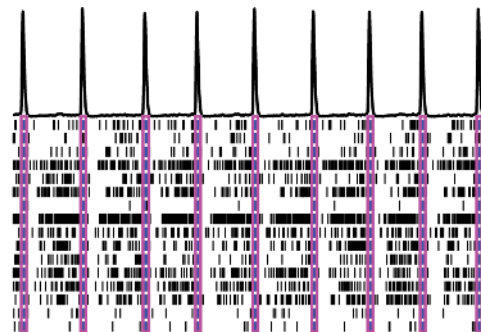
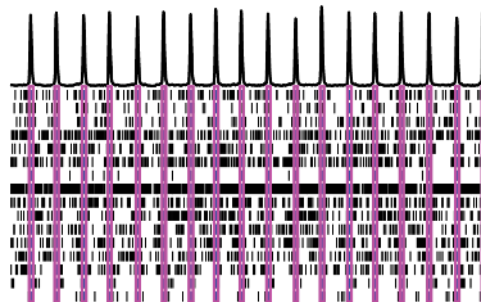
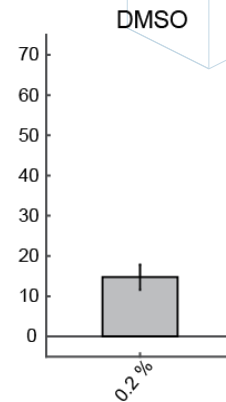
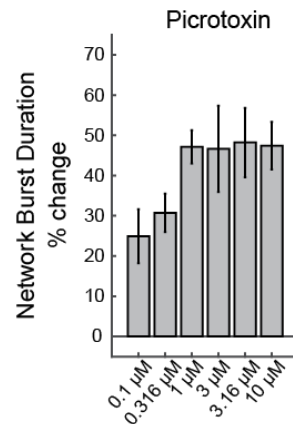
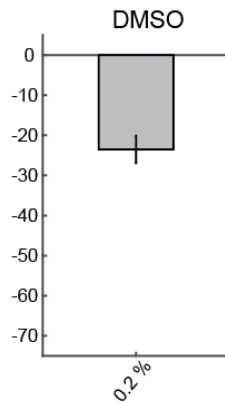
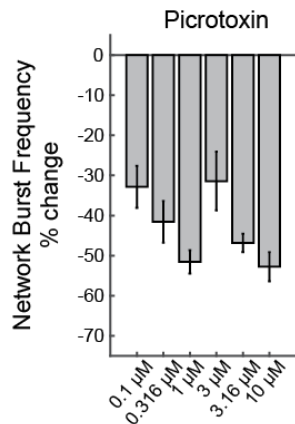
- Significant change in network activity
- Network Burst Frequency (↑)
- Network Burst Rhythmicity (↑)



MEA Pilot Study Initial Results

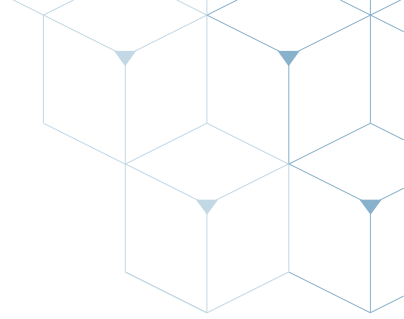
Human iPSC-Derived Neurons

- Distinct (from rodent cultures) neural network properties
- Network Burst Frequency (↓)
- Network Burst Duration (↑)



20 s

Next Steps - MEA Pilot Study



Finalize data analysis; draft and submit publication.



Share results with broader community (SOT Symposium and publication on Seizure already completed), EUROTOX session planned, additional abstracts to be submitted

Committee Areas of Focus for 2020-21



A Phase 2 in vivo study to identify biomarkers will commence to test brain region-specific or MOA-specific neurotoxicants to validate few prominent candidates from the Phase 1 study.



Model disease pathways for biomarkers using metabolomics and proteomics data via IPA-Analysis are underway.



Work will begin on MALDI-MS imaging of brain slices for biomarkers and disease models to see regional distribution of biomarkers and develop a brain correlation map.



Finalize and publish analysis of MEA data for seizure prediction.



Consolidate global outreach and engagement beyond the traditional toxicology community (via Society for Neuroscience and other targeted meetings)



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