Integration of Genomic Data Streams in an *In Vitro* Network Development Model

D A Gallegos1, C A Marable1,2, B N Chorley1, K A Wallace1, T Freudenrich1, and T J Shafer1

1. Biomolecular and Computational Toxicology Division , CCTE, ORD, US EPA, RTP, NC, USA, 2. Dept of Environmental Sciences and Engineering, UNC Chapel Hill

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David Gallegos | Gallegos.david@epa.gov | (919)-541-2623

Background And Objectives

Background: The lack of data regarding the potential DNT hazard of thousands of compounds in commerce, coupled with the time and cost limitations of present DNT guideline studies has driven efforts to develop alternative assays for characterizing the hazard. A microelectrode array-based assay (MEA) has been developed for screening and prioritization of chemical effects on neural network formation. Prior findings with this Network Formation Assay (NFA) suggest that identifying missing molecular events could help populate an adverse outcome pathway relative to neurodevelopment.

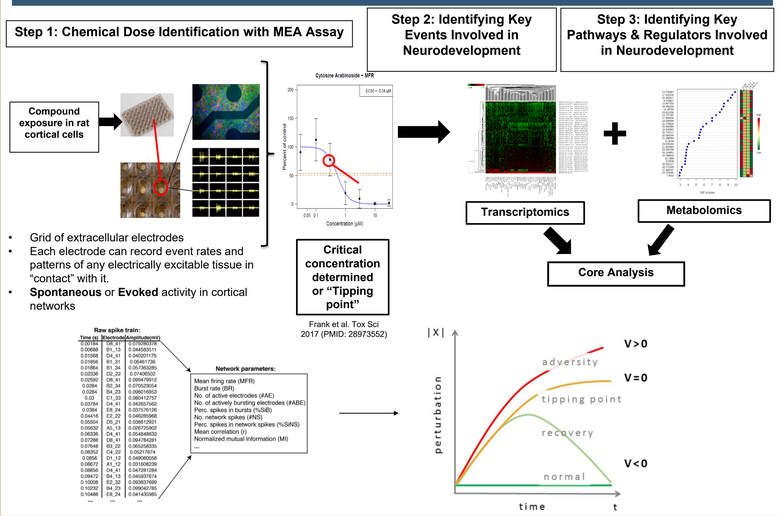
- The NFA has successfully shown tractable disruption of neuronal network development *in vitro*.
- The 24 compounds selected for –omic evaluation in this study have been shown to alter neural network formation.

Objectives:

- Utilize integrated pathway-based transcriptomic and metabolomic methods to complement NFA-observed disruptions of neurophysiological network development *in vitro*.
- Examine the observed NFA, transcriptomic, and metabolomic responses by chemical and chemical class.
- Identify key events involved in putative adverse outcome pathways leading to neural network disruption at molecular and pathway level.

Impact: ***These results provide data to build an adverse outcome pathway network based on neural network development disruption and cognate changes in cellular states.***

Method



- Grid of extracellular electrodes
- Each electrode can record event rates and patterns of any electrically excitable tissue in "contact" with it.
- **Spontaneous** or **Evoked** activity in cortical networks

MEA Assay Identifies Circuit Tipping Point Concentrations

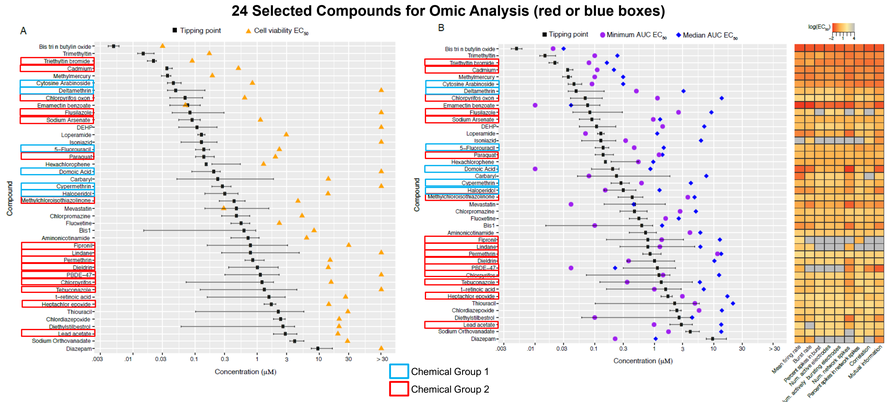


Figure 1. Comparison of tipping points to cytotoxicity and individual network parameters. Tipping points were determined for 42 compounds and compared to A) concentrations that impact cell viability (orange triangles, lower EC_{50} between total LDH and alamar blue assays) and B) the minimum (purple circles) and median (blue diamonds) EC50 value for individual network parameters based on Area Under the Curve (AUC) calculations. Heatmap shows AUC EC_{50} values for each network parameter included in tipping point determination for reference.

Combinatorial Omics Analysis Identifies Events and Pathways

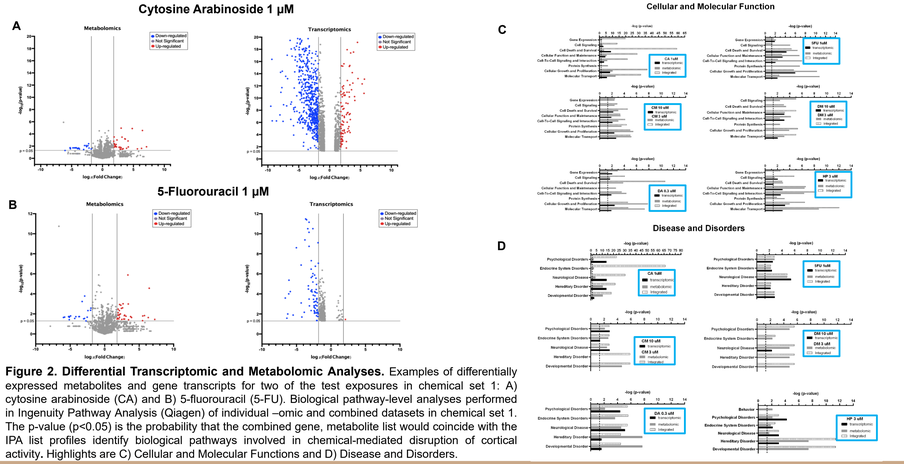


Figure 2. Differential Transcriptomic and Metabolomic Analyses. Examples of differentially expressed metabolites and gene transcripts for two of the test exposures in chemical set 1: A) cytosine arabinoside (CA) and B) 5-fluorouracil (5-FU). Biological pathway-level analyses performed in Ingenuity Pathway Analysis (Qiagen) of individual –omic and combined datasets in chemical set 1. The p-value (p<0.05) is the probability that the combined gene, metabolite list would coincide with the IPA list profiles identify biological pathways involved in chemical-mediated disruption of cortical activity. Highlights are C) Cellular and Molecular Functions and D) Disease and Disorders.

High-throughput Expansion into Broader Chemical Set

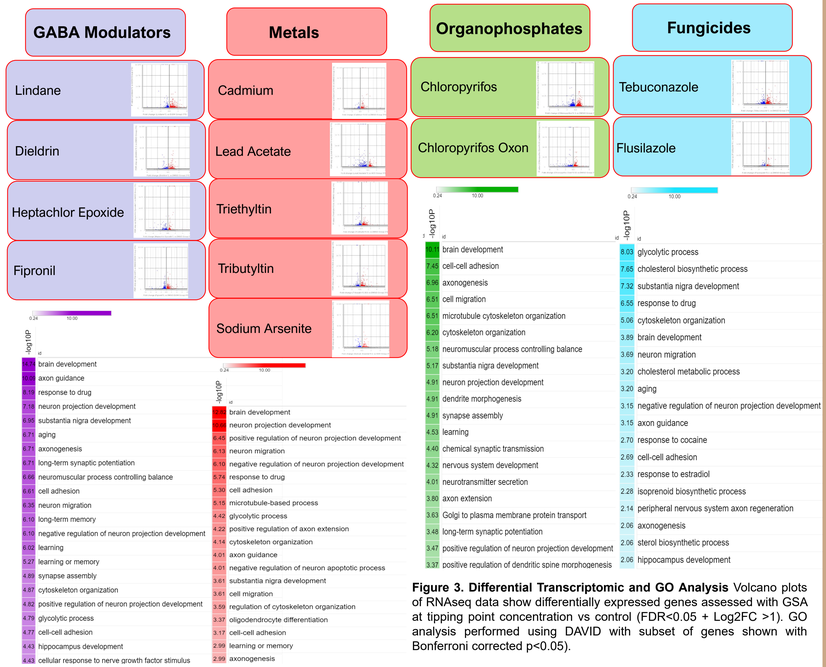


Figure 3. Differential Transcriptomic and GO Analysis Volcano plots of RNAseq data show differentially expressed genes assessed with GSA at tipping point concentration vs control (FDR<0.05 + Log2FC >1). GO analysis performed using DAVID with subset of genes shown with Bonferroni corrected p<0.05).

Conclusions and Future Directions for DNT Hazard Assessment

- A multi-omic approach identified critical pathways involved in neurodevelopment in vivo at concentrations of compounds that induce changes in network formation in vitro
- Expansion of analysis to larger chemical sets exhibits overlap in cellular processes affected by chemicals in a given class
- Future single cell RNA-seq and imaging analysis can provide increased resolution to capture complex cell-type specific features of neural network disruption. Such data will better establish early key events involved in developing adverse outcome pathways.