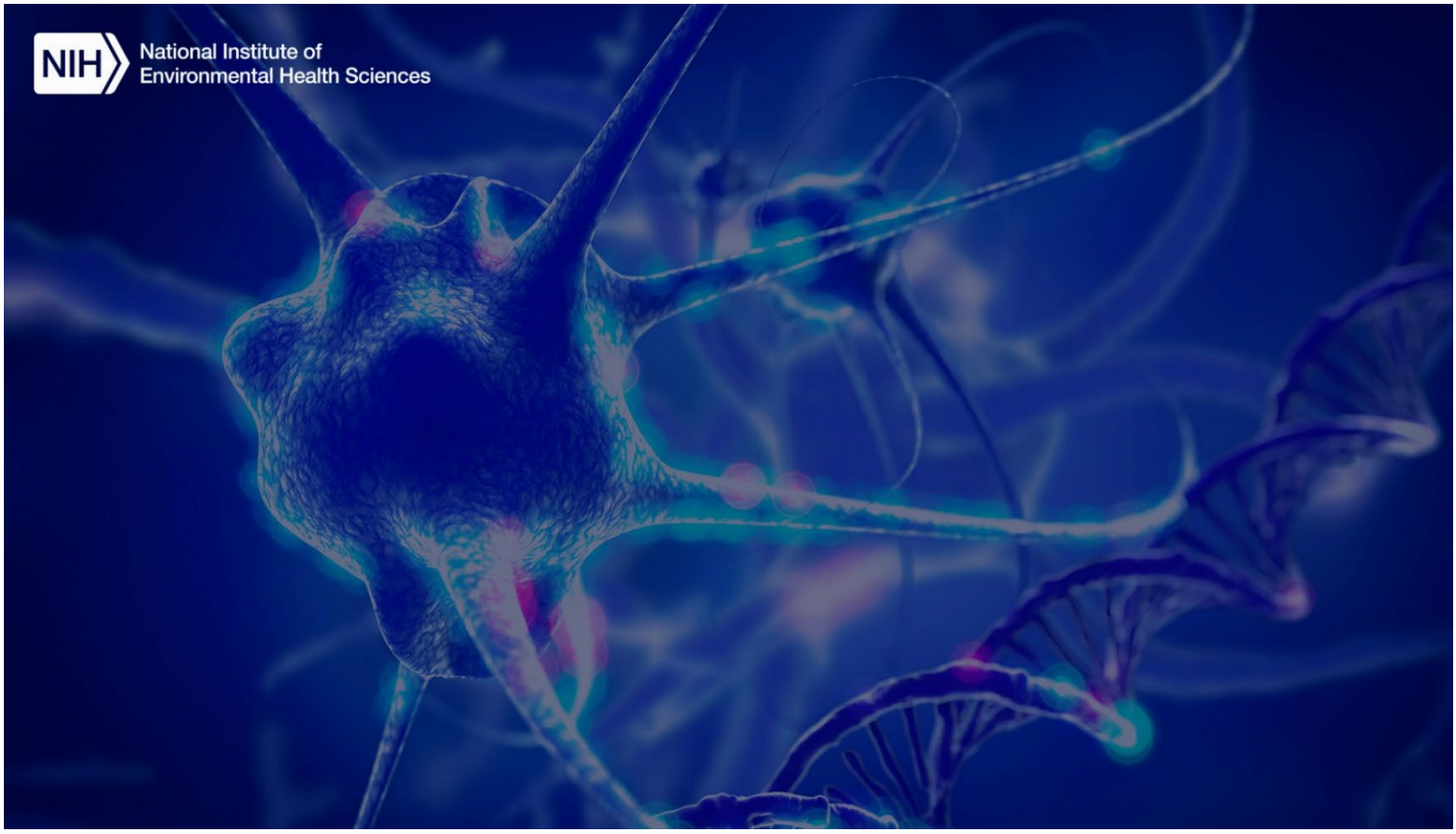

Modernizing Neurotoxicology at NIEHS: Technologies to Applications in Environmental Health Sciences

April 19-20, 2022

Workshop Report

This report was developed by Avanti Corporation in collaboration with NIEHS meeting organizers.

National Institutes of Health • U.S. Department of Health and Human Services

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Description

The National Institute of Environmental Health Sciences (NIEHS) organized a virtual workshop on April 19-20 titled “Modernizing Neurotoxicology at NIEHS: Technologies to Application in Environmental Health Sciences.” The workshop was sponsored by the NIEHS Faculty for Advancing Neuroscience (FAN). In this workshop participants explored cutting edge methods and technologies used in neuroscience and discussed their applications in environmental health sciences.

The workshop covered several topics carefully chosen for being of mutual interest across multiple divisions within NIEHS. A primary objective of this conference was to identify the gaps or challenges that need to be addressed to stimulate wide-spread adoption of cutting-edge methods and technologies in the environmental health sciences.

Workshop Overview

Meeting organizer Jonathan Hollander, Ph.D., set the stage for this meeting with a brief background on recent advances in neuroscience-related research, provided data on the current NIH investment in some of the technologies that would be highlighted at the meeting, and outlined the goals of this workshop. He stated that adoption of modern-day neuroscience approaches for toxicological research may help us better understand how toxicants can perturb neural connections within the brain, and possibly, reverse deleterious effects of toxicants by excitation/inhibition of neural circuitry.

NIEHS Director Rick Woychik, Ph.D., delivered additional opening remarks. He welcomed workshop participants and briefly discussed the different sessions. He informed participants about the request for information on research opportunities in neuroscience that was issued by NIEHS in collaboration with other NIH agencies. He also discussed how the workshop supported all three themes of the NIEHS Strategic Plan.

The goals of this workshop were to bring together a diverse group of scientists to:

- Review latest research applications of several cutting-edge tools in neurotoxicology and lessons learned.
- Identify the most pressing research questions in environmental health that would benefit from use of these new methods.
- Develop a strategy to encourage adoption of these new technologies.

The workshop was divided into four sessions, each exploring one of the following topics:

- Advances in Neuroimaging.
- In Vitro Approaches in Developmental Neurotoxicology Research.
- Chemogenetic, Optogenetic and Fiber Photometry for Advancing Neurotoxicology.
- Emerging Spatial Technologies.

Each session included four to five presentations followed by a moderated panel discussion. This report provides presentation summaries and key take away messages from each session.

Session 1: Advances in Neuroimaging

The goal of this session was to explore the application of recent neuroimaging advances to improve human health. Presentations were focused on transformative technologies such as longitudinal MRI, lipid-exchanged, anatomically rigid, imaging/immunostaining compatible, tissue hydrogel (CLARITY), De-scattering with Excitation Patterning (DEEP) and two-photon fluorescence imaging. Discussion included addressing how these technologies were aiding in further linking function, structure, and molecular endpoints for understanding the pathogenesis of neurological diseases.

Mark Shen, Ph.D., presented his research on using neuroimaging methods to study brain development of babies to identify early risk markers of autism and to reverse translate those findings in mouse models to understand pathways for potential therapeutics. His research found four brain features whose presence and severity in the first year of life could predict autism diagnosis and severity of symptoms later in life.

G Allan Johnson, Ph.D., discussed combined magnetic resonance and light sheet microscopy. He started with a history of magnetic resonance microscopy and discussed current technological developments with examples. He also discussed light sheet microscopy and stated that merging technologies will be useful in the future.

Na Ji, Ph.D., presented her work on two-photon fluorescence imaging of neurovascular dynamics and neural activity. Her work demonstrated the use of adaptive optics to allow better resolution of biological samples. She also discussed improvements to reach millisecond-scale time resolution.

Dushan Wadduwage, Ph.D., presented his research on computational neuroimaging using wide-field two-photon microscopy. He talked about using temporal focusing as an alternative to scanning two-photon microscopy. He also discussed the use of machine learning techniques to improve the speed of imaging while acquiring compressed data and shared results of simulation experiments.

Ronald Tjalkens, Ph.D., discussed his work on integrating whole-brain scanning microscopy with artificial intelligence and neural network analysis for high-throughput quantitative assessment of neurotoxicity and neurodegeneration. He gave an overview of different methods for quantifying cell numbers and phenotypes in fixed brain tissue and followed it with a detailed discussion on counting neurons in multiple sections using different methods including artificial intelligence with deep learning.

Key take-away messages

The following are key take-away messages from the discussion:

- One limitation to develop new and better deep learning methods for imaging tasks is the lack of good training data.
- *In vivo* imaging techniques may provide unique answers that are not accessible using histology-based techniques.

- Strong computational support is required for automated imaging methods. There must be a comprehensive group of players handling, digesting, and reducing data.
- NIEHS is looking for technologies to help predict diseases.
- The community needs to overcome the communication barrier between technologists and biologists.

Session 2: *In vitro* Approaches in Developmental Neurotoxicology Research

In the last two decades, scientific advances have been made which rely on human cell-based in vitro models for evaluating chemical interactions with the developing nervous system, with the aim to reduce extrapolation of in vitro developmental neurotoxicology (DNT) data to humans. This session was focused on advances in DNT modeling beginning with species-specific *in vitro* neuro stem cell development and building to a review of the complexity of the central nervous system and cell interactions in the 3-D brain organoid. Finally, the importance of including modeling of vascularization, blood brain barrier and cerebrospinal fluid to closer resemble in vivo (human) responses was discussed.

Ellen Fritsche, Ph.D., discussed the current status and gaps of the existing DNT in vitro battery. She provided an overview of the key neurodevelopmental processes targeted by the battery and examples of scientific validation assays. She also shared results of the chemical screening assays and described the gaps in the current battery of available assays.

Helena Hogberg, Ph.D., described the need for 3D neural in vitro model and her experience working with human iPSC derived 3D in vitro brain sphere model. She discussed functional assays, current challenges, and future directions for 3D in vitro models.

Guo-li Ming, MD, Ph.D., discussed her research on generation of forebrain-specific organoids from hiPSCs. She described limitations of the current organoid technology due to lack of vascularization and a using a new method for bypassing diffusion limits. She also discussed modeling BPA exposure using forebrain organoids.

Chris Hughes, Ph.D., described his experience creating vascularized micro-organs. He discussed how tissue microenvironments are complex and new 3D systems are required to capture this complexity. He also shared results of how their blood brain barrier model captures in vivo gene expression and function.

Key take-away messages

The following are key take-away messages from the discussion:

- Detection of adverse effects for different chemicals may require different *in vitro* systems that cover the same neurodevelopmental process at different timings.
- The choice of *in vitro* model system depends on the biological question being asked.
- Biostatistics should be considered for the future of *in vitro* modeling. Biostatistics for one model fitting all *in vitro* method is not ideal.
- *In vitro* assays can never be stand alone. There is an effort by EPA and European agencies to contextualize these, modeling the kinetics, the metabolism and so on to see

what ends up in the brain. It cannot be assumed that an *in vitro* test in a dish can model risk assessment of an organism.
- There is a need to develop models that can be used to study postnatal neurodevelopment.
- There is a need to harmonize protocols and lab-to-lab validation across the board to reduce variabilities of results between labs/organizations.

Session 3: Chemogenetic, Optogenetic and Fiber Photometry for Advancing Neurotoxicology

The goal of this session was to provide an overview of modern neuroscience techniques for monitoring and manipulating neural activity in vivo in the context of neurotoxicology. Presentations were focused on circuit interrogation techniques like chemogenetics, such as DREADDS, and optogenetic approaches designed to probe the neural circuit changes induced by toxicant exposures and potentially provide insight into potential therapeutics. Optical imaging approaches such as fiber photometry and miniature endoscopes were also discussed as powerful new tools that allow for unprecedented observations of neural activity in awake-behaving animals.

Timothy Allen, Ph.D., summarized how viral-based chemogenetics, multi-site chronic electrophysiology, and viral-based optogenetics are used to study neural circuitry. He presented his research on how developmental lead exposure in rats leads to widespread network dysfunction. He also discussed how excitatory optogenetics can correct aberrant synchrony in a cell- and circuit-specific way.

Jessica Plavicki, Ph.D., presented her research on using zebrafish as a model for understanding the cellular targets of PFAS-induced neurotoxicity. Using functional neuroimaging, her lab discovered that PFOS exposure caused regional increases in neuron activity in a dose- and age-dependent manner. The next step in her research is to use CaMPARI to identify specific brain regions that are affected by toxicant exposures.

Fernanda Laezza, M.D., Ph.D., shared her experience using chemogenetic approaches to rescue deficits in the reward circuit caused by exposure to deltamethrin. Her research found that novelty-induced behavior was disrupted by early-life exposure to deltamethrin. This phenotype was rescued by increasing parvalbumin interneurons firing using a chemogenetic technique.

Guohong Cui, M.D., Ph.D., discussed how multi-color fiber photometry for assessing neural circuit functions in vivo. His research showed that parallel striatal pathways collaboratively determine the dynamics and fate of actions. He also discussed how fiber photometry may be used to understand neural mechanisms of deep brain stimulations.

Key take-away messages

The following are key take-away messages from the discussion:

- Fiber photometry is not as demanding as electrophysiology in terms of data handling. Fiber photometry data is probably the easiest to analyze compared to different optical methods.

- Careful consideration should be given to the dose of clozapine-N-oxide (CNO) used for DREADD experiments, particularly with the reported side effects described in the literature. The effect(s) from the ligand can depend in part on where and how much DREADDs are expressed. Dose is not a fixed number; it's advisable to go with the lowest effective dose.
- One way to identify side effects would be to run newer DREADD ligands through the NIMH psychoactive drug screening library; the NIEHS could help with this.
- A broader problem in the field is that there is no standardization for the behavioral assays. One significant limitation to *in vivo* imaging is the cost of equipment.

Session 4: Emerging Spatial Technologies

The goal of this session was to provide an overview of current advances, challenges, and future opportunities within spatial technologies as well as the utilization of spatial technologies and research in neurotoxicology and neuroscience. Presentations were focused on various single cell and spatially resolved transcriptomics methods, as well as technical challenges that need to be overcome to obtain their full potentials. The spatial transcriptomic data analysis, understanding spatial omics dataset using visualization, machine learning and spatial statistics as well as spatial computational approaches were also covered.

Rong Fan, Ph.D., discussed the use of spatial multi-omics mapping at tissue scale and cellular level. He shared results of his work on single transcriptome mapping of the human hippocampus and generating a spatial transcriptome of normal and major depressive disorder human brains. He also discussed future directions for his research.

Ruben Dries, Ph.D., presented an overview on the methods, tools, and roadblocks in spatial transcriptomic data analysis. He described the four steps in the spatial transcriptomics pipeline: data generation, data pre-processing, analysis, and visualization. He also summarized the different tools available for each step.

Keri Martinowich, Ph.D., summarized her research on mapping the genetic risk for complex brain disorders across the spatial topography of the human dorsolateral prefrontal cortex. She presented a brief overview on the history and multiple methods of expression profiling in the human brain. She also discussed strategies for registration of pathology with gene expression.

Ansuman Satpathy, M.D., discussed the use of single cell genomics in cancer immunotherapy and neurotoxicity. He shared his research on immunotherapy antigen selection using single-cell genomics. Their results suggested that a particular antigen expressed by certain neural cells may cause neurotoxicity in patients receiving immunotherapy treatment.

Key take-away messages

The following are key take-away messages from the discussion:

- Larger reference datasets can help better understand neurotoxicity and disease state.
- Benchmarking for different technologies is very important.
- Single cell data may be useful to select antigens that may be paired together for therapy to get rid of all the tumor cells.

- AI can be powerful and critical once more data is collected. If there's enough data from imaging the same type of tissue or adjacent tissues, one day AI or machine learning may be used to fill the gaps in images, derive truly single-cell spatial transcriptome genome wide gene expression and predict gene expression between gaps.

Closing Remarks

Cindy Lawler, Ph.D., provided closing remarks at the workshop. She commented that each session in this workshop could have been a whole workshop. She stated that context was important, and the biological question was driving the adoption of new technology. She reviewed the goals of the workshop and indicated how different sessions in the workshop fulfilled those goals. She also noted that identifying the next most promising research question that would benefit from the use of cutting-edge neurotoxicology tools was a difficult task and more discussion and collaboration would be required moving forward. She acknowledged the need to raise awareness about current tools in neurotoxicology, the importance of training the next generation of scientists in using these novel tools and approaches, as well as the value of learning from the success stories of neurotoxicologists.