National Institute of Environmental Health Sciences

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

Presentation Abstracts

Virtual Workshop
April 19-20, 2022

Contents

Day One – April 19, 2022

Session 1: Advances in Neuroimaging (Chairs: Robert Sills, Kimberly Gray)

The goal of this session is to explore the application of recent neuroimaging advances to improve human health. Presentations will be 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. Discussions will be focused on how these technologies are aiding in further linking function, structure and molecular endpoints for understanding the pathogenesis of neurological diseases.

Longitudinal Neuroimaging to Study Brain Development of Infants with Neurodevelopmental Disorders

Mark Shen, University of North Carolina at Chapel Hill

Shen's research examines the early brain development of infants with various neurodevelopmental disorders (NDDs) – including autism and associated genetic conditions – enabling cross-disorder comparisons to identify early markers for these conditions. In this presentation Shen will: (1) describe how he conducts longitudinal MRI scans from 6-24 months of age in infants with NDDs, across multiple universities in the United States; (2) provide an overview of recently reported brain features that were identified in the first year of life; and (3) detail one such brain feature as an illustrative example of how he uses both longitudinal neuroimaging in children, and mechanistic studies in mouse models, to elucidate pathogenic mechanisms and inform potential clinical applications.

Combined Magnetic Resonance and Light Sheet Microscopy

G. Allan Johnson, Duke University

Magnetic resonance histology (MRH), that is MRI at microscopic resolution in fixed tissues was first suggested in 1993. The technology has seen a number of applications in toxicology. Recent technological advances now allow us to acquire images of tissue specimens at spatial resolution more that 2 million times that of clinical MRI. The method has been merged with light sheet microscopy of the same tissue providing **hi**gh-**di**mensional integrated **v**olumes with registration (HiDiver). HiDiver images are being used in neurotoxicity and neurogenetic studies providing registered, multidimensional volumes with quantitative data from meso to micro scales on connectivity, regional volumes, cytoarchitecture and cell density.

Two-photon Fluorescence Imaging of Neurovascular Dynamics and Neural Activity

Na Ji, University of California, Berkeley

Two-photon fluorescence microscopy has become the main workhorse for imaging the brains of animal models at cellular and subcellular resolution. Ji will discuss recent technological advancements in two-photon fluorescence microscopy utilizing concepts from optics and physics, such as adaptive optics, Bessel beam, and infinity mirrors, which have enabled the interrogation of neurovascular dynamics and neural activity at increasingly larger depths, higher resolution, and faster speed.

Computational Neuroimaging using Wide-field Two-photon Microscopy

Dushan Wadduwage, Harvard University

Two-photon microscopy (TPM) is the gold standard for fluorescence imaging through scattering tissue. Especially in neuroscience, point-scanning two-photon microscopy (PS-TPM) is widely used to image deep in the brain in vivo. As sequential scanned, PS-TPM however is slow. On the other hand, due to parallelism, wide-field two-photon microscopy (WF-TPM) techniques are much faster. In WF-TPM, long wavelengths enable hundreds of microns deep penetration of excitation light. But the emission fluorescence at shorter wavelengths scatters and degrades images. Small structures such as dendritic spines aren't detectable even at shallow image planes; at deep image planes, even large structures such as blood vessels appear blurred. In this work, Wadduwage discusses computational imaging techniques to overcome the limitations in WF-TPM. He first shows that a physics-based convolutional neural network (phy-CNN) can clean WF-TPM images to detect dendritic spines up to 1-2 scattering lengths deep in the mouse brain. He then introduces a structured-illuminated WF-TPM technique called DEEP, capable of imaging in vivo cortical vasculature up to 6-7 scattering lengths deep. He further speeds up DEEP by an order of magnitude using a phy-CNN. Finally, he discusses a physics-based machine learning approach called differentiable microscopy to learn optimal illumination structures, potentially speeding up DEEP by another order of magnitude.

Integrating Whole-brain Scanning Microscopy with Artificial Intelligence and Neural Network Analysis for High-throughput Quantitative Assessment of Neurotoxicity and Neurodegeneration

Ronald Tjalkens, Colorado State University

Determining numbers and phenotype of neurons and glial cells in the brain during states of disease or neurotoxicity has relied on immunostaining and light microscopy with various stereological methods for estimating cell populations within a given brain region. These methods, while accurate, are slow and typically provide information only for a single brain region. This is largely because traditional microscopy systems can only image a narrowly defined region within the field of view of the available objectives and because most analysis methods are heavily reliant on manual counting and tabulation of population and morphological data. This limits the amount and depth of information that can be obtained from a given histological specimen and requires an extended period (often many months) to acquire data sets for multiple cellular parameters from all animals in a multi-group neurotoxicity study. To address these challenges, Tjalkens has applied scanning microscopy to multi-label immunofluorescence imaging to dramatically increase both the speed of analysis (high-throughput) and the information obtained (high-content) from a given sample set. Multiple serial brain sections mounted on a single microscope slide are robotically immunostained and then scanned at high resolution by montage imaging of each entire brain section using a microscope equipped with a motorized stage. Section spacing is designed to achieve a sampling fraction encompassing an entire nucleus for accurate stereological determination of cell numbers. Scanned whole-brain images are then batch analyzed using neural networks trained to identify the features of interest, and data are obtained for multiple brain regions simultaneously by designating individual regions of interest. Hundreds of whole-brain images can be analyzed simultaneously in this fashion, decreasing the time required to complete analysis of a study from months to a few days. These methods can be applied to paraffin-embedded, frozen or CLARITY histological specimens. Not only is throughput enhanced using these methods, but the

information content obtained is greatly increased as well, because multiple cellular features can be simultaneously imaged and analyzed and changes or connections between brain regions can likewise be assessed by imaging entire brain sections. The application of these methods to neurotoxic models of Parkinson's disease will be discussed.

Session 2: In Vitro Approaches in Developmental Neurotoxicology Research (Chairs: Christopher McPherson, Shannah Witchey)

Understanding developmental neurotoxicology (DNT) is complicated by the inability to identify the underlying mechanisms responsible for adverse neurotoxic effects. This is especially difficult due to the complexity of the developing nervous system and the developmental window at the time of exposure of the organism. Examination of DNT potential of environmental compounds has historically used in vivo rodent models. Use of rodent models in DNT testing can result in scientific uncertainties extrapolating findings from rodents to humans related to temporal differences in brain development, toxicokinetics, and non-homologous behavioral tests. 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 DNT data to humans. DNT modeling is a new high priority area of focus globally and for the DNTP. This session focuses 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 is discussed.

Status and Gaps of the Current DNT in vitro Battery

Ellen Fritsche, Heinrich Heine University Düsseldorf, Leibniz Research Institute for Environmental Medicine

Testing for developmental neurotoxicity (DNT) is currently performed in rats according to the OECD/US-EPA guidelines. It is now broadly accepted that in vitro methods allow a more efficient toxicity testing for hazard identification than traditional animal experiments, concerning cost, time, and extrapolation of testing results to humans. Therefore, a DNT in vitro testing battery (DNT IVB) has been assembled under the guidance of the European Food Safety Authority (EFSA) in collaboration with the Danish- and US-Environmental Protection Agency under the umbrella of the OECD. This IVB was recently challenged with 120 compounds as a first screen to assess performance of the battery.

Fritsche will present the test systems and test methods assembled in the DNT IVB. She will explain scientific validation for some of the assays by showing cell type-specific morphology, marker expression, neurodevelopmental function and responses to pathway modulators as well as DNT compounds. She will display the outcomes of the EFSA/DK-/US-EPA screening efforts resulting in the provisional IVB performance. Finally, Fritsche will touch on battery gaps and ongoing projects aiming at closing these gaps.

In summary, this presentation aims to provide a broad overview over the current and state-of-the-art to be of the DNT IVB.

A Human iPSC-derived 3D Brain Sphere Model to Assess Developmental Neurotoxicity

Helena Hogberg, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods at NIEHS, and Johns Hopkins University Bloomberg School of Public Health

Hogberg will discuss the advantages, limitations, and challenges with the use of more complex human 3D in vitro models in developmental neurotoxicity testing.

Engineering Organoid Models for Understanding the Impact of Environmental Factors on Human Neurodevelopment

Guo-Li Ming, University of Pennsylvania, Perelman School of Medicine

Under the suspension culture conditions, human induced pluripotent stem cells (hiPSCs) have the potential to give rise to organ like structures – tissue organoids, including regionalized brain organoids. Brain organoids derived from hiPSCs have been shown to recapitulate diverse cellular compositions and maintain the general cytoarchitecture of the developing brain. These hiPSC based organoid model systems offer unique advantages in understanding molecular and cellular mechanisms governing embryonic neural development. It also provides the opportunity to understand how environmental factors, toxins or drugs might influence the neurodevelopmental processes during embryonic-fetal stages and to reveal mechanistic insights. Ming will discuss her recent work in developing unique brain organoid models to understand human brain development and the impact of potential neurotoxic environmental factors.

Modeling the Blood-Brain Barrier in a Microphysiological System platform

Chris Hughes, University of California Irvine

Maintaining a constant environment in the brain is critical to proper neuronal function, and a key component of brain homeostasis is the blood-brain barrier (BBB). The BBB is comprised of several components, that together are referred to as the neurovascular unit (NVU). These components are: endothelial cells (EC) that express numerous transporters and display extensive tight junctions and limited pinocytosis; a complex basement membrane; pericytes that wrap around the vessel; and, astrocytes that extend “foot processes” to the vessel wall and help to maintain barrier properties. These all work in concert to limit free movement of bloodborne cells and solutes into the brain while allowing and supporting delivery of key nutrients. Importantly, BBB EC also express drug efflux pumps such as P-glycoprotein (ABCB1) and MRP4 (ABCC4), which play key roles in protecting the brain from toxins. In vitro models that accurately capture the complexity and functionality of the BBB are critical if we are to understand interactions between neural tissue and bloodborne drugs and environmental toxins. Hughes and his lab have developed a microphysiological system (MPS) platform that captures this complexity, based on their previously-described Vascularized Micro-Organ (VMO). The VMO-BBB comprises a living human vascular network that connects a microfluidic artery and a microfluidic vein. The EC are induced to express a BBB phenotype and are wrapped by pericytes. Both astrocytes, which extend foot-process to the vessels, and neurons are included in the tissue, all embedded in a hyaluronan-rich extracellular matrix (ECM) resembling brain ECM. A blood substitute is perfused through the vessels and supports the underlying tissue. Single-cell sequencing confirms the BBB nature of the EC, and the vessels demonstrate

dramatically reduced permeability relative to non-BBB vessels in the same platform. The VMO-BBB provides an ideal model in which to examine drug/toxin uptake into the brain and the complex mechanisms that maintain brain homeostasis.

Day Two – April 20, 2022

Session 3: Chemogenetic, Optogenetic and Fiber Photometry for Advancing Neurotoxicology (Chairs: Jonathan Hollander, Jesse Cushman)

The goal of this session is to provide an overview of modern neuroscience techniques for monitoring and manipulating neural activity in vivo in the context of neurotoxicology. Discussions will focus 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 will also be discussed as powerful new tools that allow for unprecedented observations of neural activity in awake-behaving animals.

Viral-based Circuit-specific Tools for Understanding Neurotoxic Outcomes in the Rodent Brain, and the Great Therapeutic Potential in Targeting Disrupted Circuits

Timothy Allen, Florida International University

Great strides have been made in recent years advancing viral-based circuit-specific tools that have provided an unprecedented understating of brain-behavior relationships at the level of individual cell types and projections. Currently, the two most prominent circuit manipulation tools are chemogenetics (e.g., designer-receptors exclusively activated by designer drugs; DREADDs) and optogenetics (light-gated). These tools have revolutionized basic neuroscience and filled a glaring gap between the cellular and molecular neurosciences, and systems and behavioral neurosciences. As an example, Allen will discuss how chronic developmental lead exposure leads to massive theta (5-11Hz) hypersynchrony in the hippocampus and prefrontal cortex in adulthood, causing developmental cognitive deficits that persist throughout the life of the rats. Going beyond the simplistic notion that the cognitive deficits are “hippocampal” or “prefrontal,” Allen will then discuss the use of projection-specific DREADDs to disarticulate the contributions of multiple prominent prefrontal projection pathways through the perirhinal cortex and the nucleus reuniens, both offering direct communication with the hippocampus, but each driving different cognitive behaviors. Armed with this new circuit level understanding, he will discuss the use of channelrhodopsin to “inject” rhythmic brain activity in the hippocampus by targeting the nucleus reuniens. Stimulation of these cells, at multiple frequencies, massively reduces theta power in the hippocampus. Since hippocampal hypersynchrony is the core network problem caused by developmental lead exposure, this would seem the perfect opportunity for direct interventional approaches on the theory that we could pace-make hippocampal rhythms back to typical levels in lead exposed subjects. Allen will end with general comments about the potential benefits and caveats of using circuit-specific tools for neuroscience and environmental health sciences.

Zebrafish as Model for Understanding the Cellular Targets of PFAS-induced Neurotoxicity

Jessica Plavicki, Brown University

Epidemiological and animal model studies indicate that per- and polyfluoroalkyl substances (PFAS) congeners are toxic to multiple adaptive immune cell-types. However, little is known about how PFAS exposure affects the development or function of innate immune cells, including the resident innate immune cells of the brain, microglia. Microglia have been best studied for their canonical immune functions in resolving pathogenic and physical injuries; however, microglia also have essential, non-canonical roles in normal neural development, including sculpting neural networks through synaptic pruning. To assess whether PFOS exposure altered microglial function, Plavicki and her team performed a minor brain injury and examined the recruitment and persistence of microglia at the injury site. They found that PFOS-exposed embryos exhibited a heightened and prolonged microglial response to brain injury and that the exacerbated response was not due to changes in inflammatory cytokine signaling or an increase in cell death. Therefore, they asked whether other factors in the microenvironment, such as changes in neural activity, may be modulating microglial development and behavior. Using the photoconvertible calcium indicator CaMPARI, the researcher team performed functional neuroimaging in zebrafish larvae and observed increases in global as well as regional brain activity following PFOS exposure. Using a combination of pharmacological and optogenetic manipulations, they demonstrate that elevated neuronal activity is sufficient to exacerbate microglial responses to injury and that neuronal silencing is sufficient to rescue the observed change in microglial responsiveness following PFOS exposure. While these experiments indicate that neurons are likely important targets of PFOS-induced neural toxicity, they do not preclude microglia from being direct targets of PFOS-induced toxicity. Using optogenetics, Plavicki manipulated microglial membrane potential in PFOS-exposed fish and was able to drive microglia towards a ramified, homeostatic state and rescue the aberrant microglial behavior observed following PFOS-exposure. Together, these data indicate that both neurons and microglia are targets of PFOS-induce neurotoxicity.

Chemogenetic Approach to Rescue Parvalbumin Interneuron-related Deficits in the Reward Circuit Caused by Early-life Exposure to Deltamethrin

Fernanda Laezza, University of Texas

Growing evidence from epidemiological studies identifies early-life exposure to pyrethroids, the largest category of pesticides used in households, schools and agriculture, as a risk factor for attention-deficit hyperactivity disorder (ADHD), autism spectrum disorders, and anxiety. In support of the epidemiological studies, an animal model of early-life exposure to the pyrethroid pesticide deltamethrin (DM) recapitulates ADHD-like behavior through disruption of dopamine signaling in the nucleus accumbens (NAc), the brain region within the mesocorticolimbic pathway implicated in the human disease. Yet, the cellular and circuit mechanisms underlying the ADHD-like behavior are not fully understood.

Building on previous evidence showing that DM exerts a toxic effect on the voltage-gated Na+ (Nav) channel Nav1.1, Laezza and her team hypothesized that parvalbumin interneurons (PVIs), a subtype of inhibitory cells that control the NAc circuit output and express Nav1.1, would be particularly vulnerable to early-life exposure to DM. In support of this hypothesis, they show that action potential firing of PVIs in the NAc is reduced in the DM early-life exposure

model, a phenotype that is rescued by chemogenetic stimulation in PV-Cre mice conditionally expressing AAV-DIO-hM3D(Gq)-mCherry in the NAc. Additionally, the researchers also show that consummatory behavior for highly palatable food is disrupted in the DM early-life exposure model, resulting in DM animals consuming significantly more food than their non-exposed counterparts. Intriguingly, they find that this phenotype is rescued by increasing PVIs firing in the NAc via DREADD excitation. These results indicate that early-life exposure to DM disrupts the inhibitory control exerted by accumbal PVIs, resulting in behavioral deficits that have been previously associated with disruption of the reward circuit and ADHD-like phenotypes.

Multi-color Fiber Photometry for Assessing Neural Circuit Functions in vivo

Guohong Cui, NIEHS

Monitoring neural activities and other cellular and molecular events in awake behaving animals is crucial for understanding the neural mechanisms underlying normal behavior and neurological disorders. Fiber photometry was developed to use an optical fiber to record fluorescence signals emitted from genetically encoded fluorescent sensors expressed in the brain. In this talk, Cui will introduce the development of a spectrally resolved fiber photometry system and discuss how it is used for measuring neural activities and neurotransmitter release in vivo.

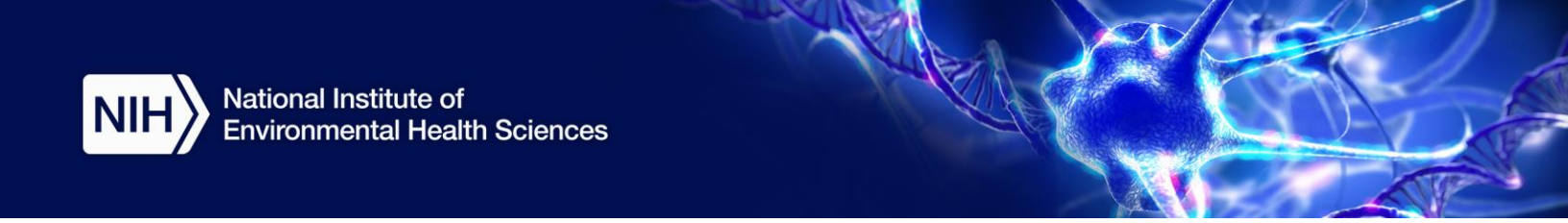
Session 4: Emerging Spatial Technologies (Chairs: Jian-Liang (Jason) Li, Benedict Anchang)

The goal of this session is to provide an overview of current advances, challenges and future opportunities within the spatial technologies as well as the utilization of spatial technologies and research in neurotoxicology and neuroscience. Discussions will focus 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 will also be covered.

Spatial Multi-Omics Mapping at Tissue Scale and Cellular Level

Rong Fan, Yale University

Despite latest breakthroughs in single-cell sequencing that revealed cellular heterogeneity, differentiation, and interactions at an unprecedented level, the study of multicellular systems needs to be conducted in the native tissue context defined by spatially resolved molecular profiles in order to better understand the role of spatial heterogeneity in biological, physiological and pathological processes. In this talk, Fan will begin with discussing the emergence of a whole new field – spatial omics in the past years and then discussing a new technology platform called DBiT-seq – microfluidic Deterministic Barcoding in Tissue for spatial multi-omics sequencing – developed in Fan's laboratory. It demonstrated, for the first time, co-mapping of whole transcriptome and a large panel of proteins with high spatial resolution directly on fixed tissue slides in a way fully compatible with clinical tissue specimen processing. First, Fan will show the application of DBiT-seq to spatial transcriptome and protein mapping of whole mouse embryo tissues that revealed all major tissue types in early organogenesis, brain microvascular networks, and a single-cell-layer of melanocytes lining an optical vesicle. Second, he will discuss spatial transcriptome mapping of FFPE tissue slides including archival human tumor specimens. Third, he will show the power of integration with single-cell RNA-seq for cell type



annotation in relation to spatial location in tissue. Finally, Fan will discuss the latest progress of DBiT as a platform technology to enable spatial epigenome sequencing (spatial-ATAC-seq, spatial-CUT&Tag, etc.) at the cellular level. The rise of NGS-based spatial omics is poised to fuel the next wave of life science revolution. Emerging opportunities and future perspectives will be discussed regarding the impact on biomarker discovery and therapeutic development.

Methods, Tools, and Roadblocks in Spatial Transcriptomic Data Analysis

Ruben Dries, Boston University

Spatial transcriptomics is a rapidly growing field that promises to comprehensively characterize gene expression and tissue organization at single-cell or subcellular resolution. Such information provides a solid foundation for mechanistic understanding of many biological processes in both health and disease that cannot be obtained by using traditional technologies. The development of computational methods plays important roles in extracting biological signals from raw data. Various approaches have been developed to overcome technology-specific limitations such as spatial resolution, gene coverage, sensitivity, and technical biases. Downstream analysis tools formulate spatial organization and cell–cell communications as quantifiable properties and provide algorithms to derive such properties. Integrative pipelines further assemble multiple tools in one package, allowing biologists to conveniently analyze data from beginning to end. Here Dries will summarize the state of the art of spatial transcriptomic data analysis methods and pipelines and discuss how they operate on different technological platforms.

Mapping Genetic Risk for Complex Brain Disorders Across the Spatial Topography of the Human Dorsolateral Prefrontal Cortex

Keri Martinowich, John Hopkins University

In this talk Martinowich will focus on ongoing projects in her lab, which aim to generate data and develop methods for single-nucleus RNA-sequencing and spatial registration of these gene expression patterns in the human brain in the context of complex brain disorders. While single cell sequencing approaches have rapidly advanced generation of molecular profiles for various cell types in the brain, a major disadvantage of these techniques is that spatial context is lost. Here, she'll describe how her lab used a combination of single-nucleus and spatial transcriptomic approaches to identify layer-enriched gene expression, spatially register single nucleus RNA-seq data and assess laminar enrichment of disease associated genes in the dorsal lateral prefrontal cortex of the human brain.

Single Cell Genomics in Cancer Immunotherapy and Neurotoxicity

Ansuman Satpathy, Stanford University

CD19-directed immunotherapies are clinically effective for treating B cell malignancies but also cause a high incidence of neurotoxicity. A subset of patients treated with chimeric antigen receptor (CAR) T cells or bispecific T cell engager (BiTE) antibodies display severe neurotoxicity, including fatal cerebral edema associated with T cell infiltration into the brain. Using single-cell genomic approaches, Satpathy and his research team identified a subset of CD19+ brain mural cells, which surround the endothelium and are critical for blood-brain-barrier integrity. These data suggest an on-target mechanism for neurotoxicity in CD19-directed therapies and highlight the utility of human single-cell atlases for designing safe and effective immunotherapies.

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