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Purpose of the Fundamental Neuroscience Working Group

The mission of the National Institute of Neurological Disorders and Stroke (NINDS), part of the National Institutes of Health (NIH), is to seek fundamental knowledge about the brain and nervous system and to use that knowledge to reduce the burden of neurological disease for all people. Research on the development, structure, and function of the normal nervous system, or fundamental neuroscience (FN), is the foundation for achieving that mission.

To address this important component of the research portfolio, NINDS convened a Fundamental Neuroscience Working Group (FNWG) of the National Advisory Neurological Disorders and Stroke (NANDS) Council to hold a series of meetings to discuss key issues and prepare a report to the NANDS Council with recommendations to inform NINDS approaches and plans to support and foster FN research.

Working Group Charge

This NANDS Working Group (hereafter referred to as the FNWG) will provide scientific guidance via NANDS Council to NINDS on how best to advance FN research.

The charge of the FNWG is to:

- **Look to the future of FN** by identifying critical gaps, key unanswered questions and new opportunities in FN research.
- **Evaluate the effectiveness and potential of current NINDS programs** to support the breadth of FN research.
- **Propose and prioritize concepts and strategies** with the potential to enhance the overall impact of NINDS FN research over the next 5-10 years.

Working Group Roster

The Working Group includes two members of the NANDS Council* and investigators with expertise in fundamental neuroscience research.

Yishi Jin*, Ph.D. (Co-Chair), *Kavli Institute of Brain and Mind, University of California, San Diego*
Timothy Ryan*, Ph.D. (Co-Chair), *Weill Cornell Medical College, Howard Hughes Medical Institute*
Bruce Bean, Ph.D., *Harvard Medical School*
David Clapham, M.D., Ph.D., *Janelia Research Campus, Howard Hughes Medical Institute*
Marc Freeman, Ph.D., *Vollum Institute, Oregon Health & Science University*
José E. García Arrarás, Ph.D., *University of Puerto Rico, Río Piedras Campus*
Alicia Dione Guemez-Gamboa, Ph.D., *Northwestern University*
Shantá Hinton, Ph.D., *William & Mary*
Oliver Hobert, Ph.D., *Columbia University, Howard Hughes Medical Institute*
Sarah Kucenas, Ph.D., *University of Virginia*
Rejji Kuruvilla, Ph.D., *Johns Hopkins University*
Wendy Macklin, Ph.D., *University of Colorado*
Kelsey Martin, M.D., Ph.D., *Simons Foundation*
Linda Richards, AO, FAA, FAHMS, Ph.D., *Washington University*
Amita Sehgal, Ph.D., *University of Pennsylvania, Howard Hughes Medical Institute*
Weiwei Wang, Ph.D., *University of Texas Southwestern Medical Center*

Process and Deliverables

The FNWG is:

- Identifying opportunities that NINDS could take to facilitate innovation and enable discoveries that are not currently addressed in FN.
- Considering specific recommendations to optimize or enhance current NINDS programs in support of the FN research mission.
- Evaluating how NINDS might support the development, refinement, dissemination and broad use of next generation technologies, approaches, or resources to open new areas of exploration.
- Presenting its draft report of findings stemming from the above charge to the full NANDS Council on September 6, 2023.

Fundamental neuroscience encompasses a broad area of research interests and levels of study and NINDS actively supports all these areas. With an eye towards new advances, the FNWG decided to focus on the areas of cellular and molecular neuroscience as the most suitable for making a meaningful impact. After establishing this focus, the subgroups of the FNWG considered key questions across seven (7) topic areas as part of the FNWG deliberations (see Table 1 below).

Table 1. FNWG-considered topic areas and subgroup members by area

Topic area	Subgroup members
1. Development	Linda Richards, Rejji Kuruvilla, Shantá Hinton
2. Genomic organization and regulation	Alicia Guemez-Gamboa, Oliver Hobert
3. Inter-tissue interaction	Marc Freeman, José García Arrarás, Sarah Kucenas
4. Metabolism	Tim Ryan, Amita Sehgal
5. Lipid stasis	Yishi Jin, Wendy Macklin
6. Atomic organization of machinery	Bruce Bean, Weiwei Wang
7. Subcellular organization of machinery	Kelsey Martin, David Clapham

Each subgroup considered the following four (4) key questions:

1. What are the critical knowledge gaps in the topic area?
2. How can we foster FN mechanistic investigation in the topic area?
3. What are the technology choke points in the topic area?
4. What are perceived funding difficulties?

Timeline

The FNWG met monthly via Zoom over 7 months in 2023. Meetings occurred:

1. January 27 (kickoff meeting)
2. February 17
3. March 17
4. April 21
5. May 19
6. June 16
7. July 27 (public webinar followed by closed session to consider input received at the public webinar)

The FNWG will complete their deliberations, including consideration of public input sent to fn@nih.gov by August 1, 2023, via email.

The FNWG will present its draft report and recommendations to the NANDS Council on September 6, 2023.

Draft Recommendations

How the nervous system develops, functions, and is maintained over the life of an organism has fascinated scientists for centuries. Despite the tremendous progress made in the intervening years to decipher arguably the most complex organ system, the FNWG identified many remaining gaps in

fundamental knowledge at the cellular and molecular scale in neuroscience, including: 1) How is metabolism regulated in the brain at the cellular and network level and how does it integrate with key signaling pathways?, 2) How do macromolecules interact with each other within cells to form higher-order conformations and how does this architecture modulate function?, 3) How do the many cell types in the nervous system interact?, and 4) How does the nervous system change over organismal lifespan and evolutionary timescales? These gaps in turn make it difficult to understand the cellular physiology in their native context, i.e., the living brain/organism.

As one of the goals of molecular neuroscience is to understand the machinery that allows all types of cells in the nervous system to orchestrate and sustain function over the life of the organism, determining the organization and functional properties of the machinery on the subcellular scale is a critical, and largely unmet goal in the field. The organization of proteins, RNAs, metabolites, and lipids within the specialized architecture of any resident cells of the nervous system is poorly understood and makes interpretation of genetic clues associated with diseases challenging. A better understanding of cell physiology, and the origins and consequences of its dysregulation, necessitates that this organization and its functional principles be understood on varying length scales, from tens of nanometers to millimeters and centimeters as well as how it varies over a physiological time scale, the developmental time scale and the lifespan of the organism. To achieve these goals, the FNWG agreed that development of new tools, together with enhancing cross-disciplinary interactions with experts in cell biology, protein chemistry, biophysics, metabolism etc., are essential to enable mechanistic leaps in our understanding of the development and function of the nervous system. In the discussions, the FNWG identified several opportunities to enable significant advancements in fundamental neuroscience research. The FNWG recommends dedicated efforts in the following areas that are poised to make transformative progress in fundamental neuroscience: macromolecular cartography; quantitative approaches to characterize protein/molecule turnover timescales and location *in vivo*; capturing and imaging cell movement and cell-cell interaction during nervous system development; and tool development for *in vivo* measurement of cellular activity. An additional recommendation calls for the incentivization of collaborations with experts outside of neuroscience paired with sustained support for technical experts and shared resources as necessary to move fundamental neuroscience research forward.

1. **Prioritize research to fill the information gap regarding the organization of macromolecular complexes within cells in the few nanometer to one micron length scale, by developing tools and research programs to create a dynamic "Macromolecular Cartography" that analyzes subsets of proteins known to be part of functional subcellular units.**

   When electron microscopy emerged as an important discipline in cell biology seven decades ago, the first images of organelles on the mesoscopic subcellular scale fueled the next generation of experiments as they provided a new intellectual framework on how to understand the cellular milieu. Three decades later, the first sets of synaptic proteins were identified through biochemical purification, but it was the identification of their geographic location within neurons that fueled discovery going forward. For much of the proteome of cells in the nervous system, however, we

lack detailed knowledge of how molecules are organized on the mesoscale. There exists an information gap regarding the organization of macromolecular complexes within cells in the few nanometers to one micron length scale. This information gap leads to a "black-box" view of the cell and leads to inferring the functional role of protein complexes (or signaling cascades/pathways) without understanding the supramolecular organization, providing an often incorrect or incomplete understanding.

As knowledge of the detailed distribution of a given molecule/protein is central to unravelling how it coordinates cellular functions, much insight will likely be gained from carrying out a detailed "Macromolecular Cartography" of brain cells. In analogy to how electron microscopy propelled thinking about cell biology, rather than simply generating a "list of molecules", we anticipate that this type of information (i.e., mapping molecules on cellular structures, and interrogating them functionally) will shepherd in a new era of molecular understanding of the nervous system.

Mapping the organization of molecules on the several nanometer scale in the context of cells that span millimeters or centimeters has traditionally been an intractable problem. In the last decade, however, advances in several complementary technologies have made accessing this level of information possible. These advances include better molecular engineering (CRISPR), affinity reagents (Halo, SNAP tags, as well custom-designed nanobody antigens), proximity ligation assays, super-resolution and expansion microscopies as well as efficient methods for combining fluorescence approaches with electron microcopy (CLEM).

Rather than going through the proteome in a scattershot manner, for macromolecular cartography we recommend that subsets of proteins be analyzed that are known to be part of functional units, with the ultimate goal of understanding how molecular organization enables function, and how this dynamically changes in different physiological contexts *in vivo*. Ideally, one would focus on studying these first in cell types where these proteins have a known physiological role amenable to deep functional analyses. Below are several examples of functional units that could be approached in this way, but numerous other such systems could be investigated:

a) Cell signaling and/or ion channel complexes at the membrane: e.g., examining the detailed subcellular distribution of G-protein-coupled receptors, and their downstream partners (G-proteins and modulators)

b) Bioenergetic and other metabolic enzymes: a detailed mapping of the distribution of key enzymes used for glycolysis and the pentose phosphate pathway and their relative positioning with respect to mitochondria or other organelles within specific subcellular milieus (dendrites, spines, axons, nerve terminals and how this compares with glial cells)

c) The fatty acid synthesis enzymes, triglyceride assembly machinery and lipases

d) The machinery for protein and organelle turnover (autophagy enzymes, proteosomes)

For all these measurements, information not only about their static subcellular localizations, but also about dynamic changes in localization is critical. This will require the development of technologies to visualize dynamic changes in trafficking and in molecular interactions within cells.

2. **Enable quantitative approaches to characterize protein/molecule turnover timescales and location *in vivo* to advance understanding of molecular interactions and how they contribute to cellular stability and plasticity.**

   Cells in the nervous system, particularly neurons, turn over very little or not at all. How these cells integrate and stabilize different molecules and macromolecular complexes remains a mystery. To understand how cells and circuits are stabilized or exhibit structural plasticity, and the role of protein-protein interactions in these events, there is a need for rich spatial quantitative protein information across developmental stages, across cell types, and during cell-cell signaling. Recently the first estimates of how a neuronal protein, PSD95, turns over in different brain regions has become possible using a combination of CRISPR tagging and self-labeling tags (Halo) using pulse chase fluorescent labels of different colors. This approach demonstrated that it is possible to obtain estimates of local protein turnover time in a spatially defined fashion. We encourage further technical development in this area that will allow this approach to be deployed as broadly as possible. Integrating this information with macromolecular cartography will enhance fundamental knowledge on cellular stability and plasticity.

3. **Support approaches for capturing and imaging cell movement and cell-cell interaction during development of a (any) nervous system.**

   The cellular and physiological complexity of the nervous system is remarkable and daunting. Understanding how the development of neurons, glia, vascular, immune cells, and other cells is coordinated across spatial- and timescales to generate this tissue is an unmet and crucial goal. A much more comprehensive understanding of the cellular mechanisms that drive development, coordinate morphogenesis by signaling to other cells, and ultimately assemble any neural tissue is essential.

   The Working Group noted that this same understanding is required of the peripheral nervous system, which has been historically understudied. Its connection to the central nervous system and integration of all sensory modalities make it an intriguing system in which to study how these two halves of the nervous system develop and function and how they differ in their regenerative and plasticity capacities. Improved understanding of the peripheral nervous system will also provide new insight into how innervation is coordinated with organogenesis during development and how the nervous system controls bodily functions.

   Additionally, the Working Group emphasized that researchers who work with model systems, simple, or non-canonical systems, are poised to rapidly elucidate key early developmental processes (e.g., neural crest migration, developmental apoptosis) in the central and peripheral nervous systems, and use those findings to illuminate evolutionarily conserved mechanisms in humans. Visualizing developmental processes, such as neural crest migration, developmental

apoptosis, axon pathfinding/branching, circuit formation and formation of structures like the blood brain barrier, particularly *in vivo*, is necessary to provide the foundation for hypothesis-driven mechanistic studies on how these processes are regulated. The Working Group also recognized that while techniques such as single cell-transcriptomics have vastly improved our understanding of neural development, these studies should be followed by mechanistic studies to define the specific molecular pathways that underlie the development of neuronal populations and their interactions with other cell types.

4. Ensure adequate resourcing of tool development for *in vivo* measurement of cellular activity.

To make fundamental discoveries about the nervous system and gain mechanistic insight into how it develops, functions, and is maintained, novel tools need to be developed to give researchers unprecedented access to the cellular physiology of the *in vivo* brain. The toolkit for imaging neuronal activities has expanded significantly in the neurosciences during the last decade with the development of genetically encoded sensors for Ca^{2+}, cAMP, and voltage as well as several fast and slow acting neurotransmitters. The development of GCaMP for imaging neuronal activity is a successful example of close collaboration between tool developers and tool users that was critical for rapid improvement in GCaMP design and its validation and *in vivo* use in many experimental models. These imaging tools need to be complemented by “ground-truth” electrophysiological recording of activity in developing brains that provides greater fidelity than EEG measurements. This is currently technically challenging and requires the development of new types of recording apparatus and techniques. Moreover, as described below, there are very limited tools/sensors for many other processes across whole cells or cellular compartments. The FNWG recommends that greater resources—time and financial support—should be invested to foster interdisciplinarity between neuroscientists and biochemists, cell biologists, metabolism experts, engineers, geneticists and researchers working on non-canonical model organisms. Collaborations with other Institutes, such as the National Institute of Biomedical Imaging and Bioengineering (NIBIB), would likely be beneficial and necessary in such a tool-building effort. The FNWG further considers that developing transformative new tools to track metabolites and lipid flux could be essential for numerous fundamental molecular breakthroughs.

4.1. Encourage the development of tools for tracking metabolites in the brain.

Metabolism is closely intertwined with cognitive states, human brains are acutely sensitive to interruptions in fuel supply, and molecules that were previously thought of as simple bioenergetic intermediates are now also appreciated to be crucial signaling molecules. Altered brain metabolism is also considered a precursor to the eventual onset of neurodegeneration. Yet metabolism in neural tissue is much less understood than in other tissues, owing in part to the architectural and cell type complexity in the brain and to the relative inaccessibility of the nervous system. Thus, standard metabolic measurement techniques are difficult to deploy and interpret in brain tissue. An area that is ripe for technical advances is the development of genetically encoded sensors for a suite of key metabolites, which, when successful, will allow subcellular tracking of metabolites of

individual cells in intact or semi-intact brains. Although some sensors for glycolytic metabolites (glucose, ATP, pyruvate, lactate, NAD+) have been developed, these are limited in scope, and in general existing tools require significant optimization to allow for both accurate quantification and unambiguous interpretation of signals. In addition to the metabolites mentioned above, the toolkit should be expanded to allow real-time detection of metabolites that are known to serve as critical intersection points between anabolic and catabolic pathways, or cell signaling pathways. Examples include: phosphogluconate, now thought to provide feedback signals between glycolysis and the pentose phosphate pathway; NADPH, a critical regulator of redox control; and intermediates of the TCA cycle that lie at the intersection of anaplerosis and cataplerosis (citrate, fumarate, a-ketoglutarate, oxaloacetate).

4.2. Encourage the development of *in vivo* sensors for tracking lipids in cellular compartments.

Like metabolites, lipids play essential roles not only in building cellular architecture but also for cellular signaling and repair. Identifying lipid species and composition in a given cell type is challenging. Recent technological advances in spatial lipidomics offers some hope and should be encouraged. Neurochemists have been developing photoflippable (i.e., switch on and off via light) lipids via the use of azobenzene bonds, and driven to specific cellular localization via SNAP-tag and “click chemistry”. This can potentially begin to address the unknown mechanisms underlying intracellular lipid signaling. Tools for tracking lipid flux *in vivo* with subcellular resolution are limited. Available sensors for several phospholipids (PIPs, PS) are somewhat useful, and may be optimized and expanded to other lipid species. Fluorescently tagged cholesterol derivatives are currently available for fixed tissue, but novel cholesterol derivatives are needed for *in situ* analysis, for example alkyne-cholesterol analogs that can be labeled with fluorophores by click chemistry. As most lipid sensors have only been tested *in vitro* or in cell lines, close collaborations between neuroscientists and other chemists would likely be beneficial and necessary in such a tool-building effort.

5. Promote interdisciplinary team science and collaborations with technical expertise across diverse disciplines.

Much of the proposed research work would require team science, involving neuroscientists, chemists, engineers and others who can develop novel approaches to understanding the development and function of the nervous system. In this endeavor, sustained support for technical experts, such as scientists engaged in tool development or involved in supporting science infrastructure facilities, was also identified as key to ensuring the continued success of fundamental neuroscience research. Such investigators can identify crucial technical and other non-hypothesis driven approaches to understanding the nervous system and they need to be supported both by funding at the NIH and by recognition at their home institutions. Novel collaborations with experts outside of neuroscience need to be incentivized by, for example, developing shared funding mechanisms with other NIH Institutes and expanding the use of high-end equipment core facilities.