Modeling Basic Mechanisms of Brain Aging and Alzheimer’s Disease

*In Vitro and In Silico*

*A virtual workshop organized by the National Institute on Aging*

April 27-28, 2022

Virtual Meeting

August 9, 2022

This meeting summary was prepared by Rose Li and Associates, Inc., under contract to the National Institute on Aging (47QRAA19D00D7). The views expressed in this document reflect both individual and collective opinions of the meeting participants and not necessarily those of NIA. Contributions to this meeting summary by the following individuals is gratefully acknowledged: Gina Castelvecchi, Elizabeth A. Finch, Kayla Harr, Kristen Brennand, Dana Carluccio, Mark Cookson, Yuri Dabaghian, Antonio del Sol, Amanda DiBattista, Anna Greenwood, Henry T. Greely, Chelsea Haakenson, William Lytton, Cian O’Donnell, Rudolph Tanzi, Giuseppina Tesco, Li-Huei Tsai, Tracy Young-Pearse.
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Acronym Definitions

1D 1-dimensional
2D 2-dimensional
3D 3-dimensional
AD Alzheimer’s disease
ADRD Alzheimer’s disease and related dementias
AMP AD Accelerating Medicines Partnership® Alzheimer’s disease
ANN artificial neural network
APOE Apolipoprotein E
APP amyloid precursor protein
ATAC Assay for Transposase-Accessible Chromatin
BBB blood-brain barrier
Cas9 clustered regularly interspaced short palindromic repeat-associated protein 9
CRISPR clustered regularly interspaced short palindromic repeats
CRISPRa clustered regularly interspaced short palindromic repeat activation
CRISPRi clustered regularly interspaced short palindromic repeat interference
DEG differentially expressed gene
DNNs deep neural networks
ECM extracellular matrix
eQTL expression quantitative trait loci
FAD familial Alzheimer’s disease
GSEA gene set enrichment analysis
GWAS genome-wide association studies
hESC human embryonic stem cell
hiPSC human induced pluripotent stem cell
hNPC human neural precursor cell
iBBB induced blood-brain barrier
iFBN induced forebrain-type cortical neurons
iMGLs iPSC-derived microglia
iNDI iPSC Neurodegenerative Disease Initiative
iNs interneurons
iPSC induced pluripotent stem cell
IRB institutional review board
LOAD late-onset Alzheimer’s disease
MAP Memory and Aging Project
MGLs microglia
MODEL-AD Model Organism Development and Evaluation for Late-Onset Alzheimer’s Disease
MSM multiscale modeling
NCI non-cognitively impaired
NFT neurofibrillary tangles
NF-κB nuclear factor kappa B
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<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>NIA</td>
<td>National Institute for Aging</td>
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<td>NIH</td>
<td>National Institutes of Health</td>
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<td>NPC</td>
<td>neural precursor cell</td>
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<td>PBMC</td>
<td>peripheral blood mononuclear cells</td>
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<td>PSEN1</td>
<td>presenilin 1</td>
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<tr>
<td>Ptau</td>
<td>phosphorylated tau</td>
</tr>
<tr>
<td>ROSMAP</td>
<td>Religious Order Study and Memory and Aging Project</td>
</tr>
<tr>
<td>scRNA-seq</td>
<td>single-cell RNA sequencing</td>
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<td>SNP</td>
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Executive Summary

The Modeling Basic Mechanisms of Brain Aging and Alzheimer’s Disease *In Vitro* and *In Silico* workshop was convened by the National Institute on Aging’s (NIA) Division of Neuroscience (DN) to gain insight into major directions in the fields of *in vitro* and *in silico* modeling of healthy brain aging and AD and to articulate important research questions. *In vitro* and *in silico* modeling offer opportunities to conduct complex studies of the underlying mechanisms driving brain aging and dementias. Yet to pursue these opportunities, researchers must grapple with a set of interrelated biological, computational, and ethical challenges. This workshop explored the state of the science and identified opportunities and challenges in key areas, such as the development of *in vitro* models that better recapitulate *in vivo* conditions and phenotypes, pathways to foster collaborations across experimental and computational modeling, and the potential to leverage existing research structures to evaluate evolving neuroethical considerations related to *in vitro* and *in silico* models.

The two-day workshop featured three sessions: (1) Highlighting the Opportunities and Challenges in Modeling Basic Mechanisms of Brain Aging and AD *In Vitro*, (2) Exploring the Challenges and Potential Value of Modeling Basic Mechanisms of Brain Aging and AD *In Silico*, and (3) Consideration of the Ethical Implications of Modeling Mechanisms of Brain Aging and AD *In Vitro* and *In Silico*. Discussions of the key themes followed the presentations for each session, and the workshop concluded with an overarching discussion of research gaps and opportunities for progress that emerged throughout the workshop.

**Session I: Highlighting the Opportunities and Challenges in Modeling Basic Mechanisms of Brain Aging and AD *In Vitro***

Session I Chair Dr. Li-Huei Tsai moderated presentations and discussion related to *in vitro* modeling of brain aging and AD mechanisms. Dr. Tsai began the session with an overview of the history of technological developments that have advanced modeling and understanding of AD neuropathogenesis. Dr. Tracy Young-Pearse continued by outlining her laboratory’s efforts to use large collections of human induced pluripotent stem cell (hiPSC)-derived neurons (iNs) from aging cohorts to capture the heterogeneity of AD and healthy brain aging and elucidate the molecular pathways underlying risk and resilience to AD. Dr. Tsai continued by outlining co-culturing models to understand cell type-specific contributions of *APOE4* to AD phenotypes. Dr. Giuseppina Tesco further detailed the challenges and opportunities for the use of a 3D *in vitro* model built upon silk-collagen scaffolds that enables long-term co-culturing of neurons and astrocytes to model familial AD (FAD). Following the presentations, the speakers discussed strategies to improve *in vitro* models’ recapitulation of *in vivo* systems, approaches to using *in vitro* models to identify correlational and causal AD biomarkers, and how the integration of multiscale model data can provide additional insights into the pathology of AD.

After the first Session I discussion session, Dr. Mark Cookson provided background on two NIA resources to support the identification of gene variants associated with AD: the iPSC Neurodegenerative Disease Initiative (iNDI) and *in vitro* expression quantitative trait loci (eQTL) analysis of variants in noncoding regions associated with AD. Dr. Kristen Brennand expanded on

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this background, describing CRISPR-based approaches to study the impact of brain-disease associated eQTLs, alone and in combination, to work toward resolving the complex risk architecture of highly polygenic neurological diseases. Dr. Rudolph Tanzi then outlined multiple 3D co-culture models incorporating various neuronal, glial, and immune cell types to model the pathogenic cascades and phases of AD. Following these presentations, the speakers discussed: (1) the utility of in vitro AD models in drug screening and development, (2) the importance of studying AD in the context of dysregulated pathways rather than individual gene variants, (3) research strategies for including environmental and lifestyle factors in AD models, (4) research strategies to model aging in vitro, (5) strategies for conducting research at a larger scale, and (6) the importance of collaboration and improved communication between experimentalists and computational researchers.

Across both the presentations and discussion, participants highlighted key gaps and opportunities related to in vitro modeling of AD and aging, including but not limited to the following:

**Enhancing the Complexity of In Vitro AD Models**
- How can 3D co-culture models be used to further understand the cell type-specific contributions of mutations to AD phenotypes?
- How can the in vivo behaviors of microglia (MGLs) be recapitulated in vitro?
- How can additional cell types and peripheral factors be incorporated into in vitro models of AD and aging?
- How can environmental factors that affect AD risk be modeled in vitro?
- What factors contribute to the phenotypic heterogeneity in individuals with AD neuropathologies?

**Developing In Vitro Models of Healthy Aging**
- Do hiPSC-derived neuronal cell types retain any latent aging signatures?
- Which hallmarks of aging are preserved in hiPSC-derived cells?

**Identifying Applications of In Vitro AD Models**
- How can in vitro models of AD be used to identify useful disease biomarkers?
- How can analysis of in vitro models be used to understand complex synergistic contributions of eQTL variations to AD risk?
- How can in vitro 3D AD models be used to accelerate therapeutic screening and development of AD preventatives and treatments?
- What disrupted pathways in AD could serve as druggable targets for prevention and treatment of the disease?

**Using Large Datasets Effectively**
- How can large datasets from human brain studies be used to validate in vitro models of AD and aging?
• How can experimentalists and computational researchers form successful, long-term collaborations for modeling AD and aging?

**Session II: Exploring the Challenges and Potential Value of Modeling Basic Mechanisms of Brain Aging and AD in Silico**

Session II Chair Dr. William Lytton moderated presentations and discussion related to *in silico* modeling of basic mechanisms of brain aging and AD. Dr. Lytton began the session with an overview of the types of *in silico* models being applied to brain function and dysfunction. He then presented his own work illustrating applications of multiscale modeling (MSM) to provide an integrated description of different spatial and temporal scales of neural organization, including recent advances in the use of digital twin models to simulate complexities of brain structure and function in aging and AD. Dr. Cian O’Donnell then discussed implications of the fundamental concepts of convergence and redundancy for modeling brain circuitry and understanding brain disorders as well as the strengths and limitations of using deep neural networks to model brain function and dysfunction. Dr. Antonio del Sol described several applications of MSM to regenerative medicine and to brain aging and disease that illustrate the utility of this approach for illuminating biological mechanisms and that highlight the synergy between experimental and computational efforts. Dr. Yuri Dabaghian described a computational modeling approach that uses topological maps as a framework for understanding how the hippocampus creates an internal representation of space, particularly through mechanisms at the circuit, synchronization, and ensemble levels, and how impairments of these mechanisms can lead to spatial learning and memory deficits in AD. Dr. Anna Greenwood shared the work of Sage Bionetworks to build and maintain an openly accessible data sharing infrastructure that distributes data tools and research resources emerging from NIA-funded initiatives. Following the presentations, the speakers discussed the importance of sharing computational tools, incorporating induced pluripotent stem cell (iPSC) model data into data explorers, applying MSM tools to brain aging and AD, and developing *in vitro* and *in silico* modeling of healthy brain aging.

Across both the presentations and discussion, participants highlighted key gaps, challenges, and opportunities related to modeling of basic mechanisms of brain aging and AD *in silico*, including but not limited to the following:

**Sharing Computational Modeling Tools**

• What resources, tools, and forums are available or could be developed to facilitate sharing of *in silico* models and modeling tools with experimentalists and other computational researchers?

**Incorporating iPSC Model Data into Explorers**

• What is needed to build an *in vitro* data sharing infrastructure for *in vitro* model systems that is equally findable, accessible, interoperable, and usable by both experimental and computational researchers?
Applying MSM tools to Brain Aging and AD

- How can multiscale and other modeling approaches be used to enable a more comprehensive view of the mechanisms underlying brain aging and AD phenotypes?
- How can computational approaches guide the design of novel therapeutic approaches to treat aging-related neurodegenerative diseases?
- What are the most appropriate in vitro systems for modeling dysregulation of biological processes associated with brain aging and disease and for testing potential therapeutic strategies to reverse the aging and AD phenotype?
- How can emerging multi-omics data be leveraged and integrated to develop more accurate in silico models?
- How can initiatives that support sustained engagement and collaboration between experimental and computational researchers advance the development of MSMs and other computational models of brain aging and AD?

Developing In Vitro and In Silico Modeling of Aging

- Can computational models incorporate cellular level hallmarks of healthy brain aging for different neural types or other types of data to serve as a guideline for experimentalists trying to recapitulate aging in a dish?

Session III: Consideration of the Ethical Implications of Modeling Mechanisms of Brain Aging and AD In Vitro and In Silico

Dr. Insoo Hyun presented approaches to reinforce existing structures in research ethics to address both immediate and future ethical challenges of modeling mechanisms of brain aging and AD in vitro and in silico, such as implementing future-oriented informed consent practices and ensuring rigorous ethical oversight of preclinical studies. Henry Greely discussed further ethical considerations that merit the attention of researchers, including data privacy and ownership and potential risks presented by the public reception and interpretation of organoid research. Following the presentations, the speakers discussed how to communicate with donors about in vitro and in silico models, establish a robust preclinical evidence base, ensure that the results of studies using in vitro models are both reproducible and generalizable, and ethically distribute resources to support high-impact research.

Across both the presentations and discussion, participants highlighted key gaps and opportunities related to the ethical implications of modeling mechanisms of brain aging and AD in vitro and in silico, including but not limited to the following:

Fostering Research Oversight

- How can existing ethics and efficacy review structures be enhanced to ensure rigorous oversight of in vitro and in silico studies?
- What preclinical evidence base is needed for research conducted in organoid models to ethically reach first-in-human clinical trials?
**Communicating with Donors and the Public**
- How can informed consent language address data sharing and privacy while ensuring that specimens can ethically and legally be used in future brain organoid research?
- How can researchers mitigate potential misinterpretations of organoid research among donors and the public?

**Maximizing Research Applicability and Impact**
- How many cell lines and what extent of genetic diversity are needed to ensure that *in vitro* and *in silico* studies fulfill the ethical obligation to generate reproducible, generalizable results that benefit human health?
- How can funding agencies, peer reviewers, and researchers ethically allocate resources and efforts to support research with the greatest promise and potential impact?

**Session IV: Final Panel Discussion and Wrap-Up**
Dr. DiBattista moderated a final discussion related to overall AD and aging research gaps and opportunities. Speakers identified gaps and opportunities, including but not limited to:

- Which scales are appropriate for studying aging *in vitro*?
- What hallmarks and biomarkers should be used to define aging *in vitro*?
- How can the research community address the current limitations of *in vitro* models of AD?
- How can communication and collaboration between experimentalists and computational researchers be improved?
- What are the advantages and disadvantages of using hiPSCs and directly converted neurons to study aging?
Meeting Summary

Welcome and Opening Remarks
Amanda DiBattista, PhD, Division of Neuroscience, National Institute on Aging
Melinda Kelley, PhD, Acting Deputy Director, National Institute on Aging

NIA leadership thanked workshop organizers, speakers, panelists, and session chairs for their participation and outlined the three main topics for the workshop: (1) modeling brain aging and AD \textit{in vitro}, (2) bridging different \textit{in vitro} models using \textit{in silico} approaches, and (3) identifying ethics and policies surrounding \textit{in vitro} AD research. The key questions addressed in this workshop were:

1. What can \textit{in vitro} “AD disease-in-a-dish” or population level “village-in-a-dish” approaches tell us about underlying mechanisms driving brain aging and dementia?
2. To what extent are human cell reprogramming approaches like iPSCs and brain organoids reflective of their donors’ age, neuropathology, and cognitive status?
3. How might \textit{in vitro} research intersect with simulated models and computational approaches to understand mechanisms driving brain aging and AD \textit{in silico}?
4. How can existing research structures be leveraged to evaluate the neuroethical considerations accompanying \textit{in vitro} and \textit{in silico} modeling of brain aging and AD?
5. What are the advantages and disadvantages to using \textit{in vitro} and \textit{in silico} methods to model basic mechanisms of brain aging? What is needed to move the field forward?

Session I: Highlighting the Opportunities and Challenges in Modeling Basic Mechanisms of Brain Aging and AD \textit{In Vitro}
Session Chair: Li-Huei Tsai, PhD, Massachusetts Institute of Technology

Neuroscientific discoveries have historically driven subsequent waves of technological development that have advanced fundamental understanding of AD pathogenesis. In 1906, Alois Alzheimer’s description of the first AD patient drove major technological developments in histology. In 1985, tau and amyloid became recognized as important components of tangles and plaques in the brain, leading to the development of new biochemical tools. In the 1990s, researchers identified mutations associated with late-onset AD (LOAD) and familial Alzheimer’s disease (FAD), and genetic tool development accelerated. Human embryonic stem cells (hESCs) were derived in 1998 and human induced pluripotent stem cells (hiPSCs) were derived in 2007, and AD researchers developed and assessed protocols for stem cell culturing. After the first AD genome-wide association study (GWAS) in 2009, development of functional genomics tools accelerated. For example, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) genome editing system was developed in 2012, enabling targeted gene editing for \textit{in vitro} disease modeling. More recently, single-cell transcriptional profiling now facilitates the study of individual cells in more complex \textit{in vitro} models and enables benchmarking of these models against human systems to assess recapitulation of \textit{in vivo} phenotypes.
hiPSC models continue to increase in cellular and architectural complexity. Diseases can now be studied in 3D co-cultures of multiple cell types that better recapitulate in vivo conditions and phenotypes. These co-cultures are also useful for understanding cell-cell interactions across multiple cell types. New developments in in vitro modeling will enable new discoveries that in turn will drive the technological development of more complex in vitro model systems. Session I presentations and discussions focused on addressing the following outstanding questions related to in vitro modeling of brain aging and AD:

1. What are the advantages and disadvantages of currently available tools and resources, and how can these be improved to study AD and aging?
2. What strategies can help make findings from in vitro models more generalizable to in vivo observations?
3. What are the opportunities for using in vitro approaches to advance the fields of brain aging and AD/ADRD?
4. What scientific questions are best addressed through in vitro modeling?

Interrogating Alzheimer’s disease pathogenesis using iPSCs: delineation of aspects of biology captured and not captured

Tracy Young-Pearse, PhD, Brigham and Women’s Hospital and Harvard Medical School

AD studies typically compare AD to non-cognitively impaired (NCI) individuals in experimental systems, but the heterogeneity in genetic risk and protective factors, neuropathological burden, and cognitive trajectory that exists within both groups is not adequately captured by these types of comparative studies. Dr. Young-Pearse used hiPSC to begin to capture person-specific heterogeneity across the population. hiPSC were derived from more than 50 individuals enrolled in either the Religious Orders Study (ROS) or Memory and Aging Project (MAP) cohorts. The hiPSC lines were generated from peripheral blood mononuclear cells (PBMCs) and subsequently differentiated into iNs for further analyses. She showed that using the reductionist system of monolayer monocultures enables the study of molecular processes directly affected by genetic variation, and allows for the quantification of biological process affected by this variation in a person-specific manner.

Association analyses identified quantifiable congruence in RNA and protein expression between donor-matched iNs and brain tissue, indicating that iN cultures are partially recapitulating RNA and protein expression profiles. Differentiation of the iPSC cohort to astrocyte fate showed that astrocyte RNA and protein expression also were partially congruent with brain tissue RNA and protein expression.

To determine the level of concordance between altered pathways and protein expression in iNs and brain tissue, Dr. Young-Pearse’s research group used categorical analysis to identify differentially expressed RNA and protein modules originally detected in brain tissue. Comparisons of AD to low and high pathology NCI iNs identified eight differentially expressed protein modules previously identified in brain tissue. Of those eight, four were validated in brain tissue from the same individuals. These findings indicate that IN culture partially
recapitulates post-mortem brain tissue expression profiles; study of other neural cell types will likely identify other congruencies between hiPSC-derived monolayer cultures and post-mortem brain tissue.

Certain aspects of Aβ and Ptau levels detected in iNs are associated with Aβ plaque and NFT pathologies observed in donor-matched post-mortem brains. While no association was identified between overall Aβ levels in iNs and post-mortem brain Aβ plaque or NFT scores, certain Aβ ratios (e.g., Aβ40/total Aβ, Aβ42/total Aβ) and ratios of certain Ptau species (e.g., p202, 205TAU(major)/TAU, p202, 205TAU(HMW)/TAU) were associated with higher brain Aβ plaque and NFT scores, respectively. In addition, levels of long to short Aβ and aggregated phosphorylated tau were associated with global cognition scores from the respective donors at their last clinic visit. These associations are likely being driven by overall polygenic risk, as long to short Aβ were associated with LOAD polygenic risk score. With more complex co-culture and 3D models, researchers will be able to capture more aspects of molecular programs involved in AD pathogenesis and gain a better understanding of the effect of heterogeneity on global cognition.

To determine whether hiPSC-derived neurons retain a latent signature of aging, PBMC-derived hiPSCs from 53 individuals in the ROSMAP cohort were analyzed using gene set enrichment analysis (GSEA), which identified associations between age of PBMC collection and expression of certain gene sets. Specifically, iNs derived from older donors had higher expression levels of genes involved in oxidative phosphorylation, a hallmark of brain aging. In addition, age of demise was positively correlated with higher expression of genes involved in oxidative phosphorylation in brain tissue from the same individuals. Comparison of hiPSCs derived from PBMCs collected at different ages from the same donor will enable further investigation into a possible latent signature of aging.

Modeling multicellular interactions in AD brains using induced pluripotent stem cells

Li-Huei Tsai, PhD, Massachusetts Institute of Technology

The Apolipoprotein E4 (APOE4) allele is associated with an increased risk of LOAD. Understanding the cell-autonomous and non-cell-autonomous mechanisms mediating the impacts of APOE4 requires co-culturing of multiple neural cell types in 3D in vitro models that mimic blood-brain barrier (BBB) dysfunction and neural cell type interactions observed in vivo in AD brains.

The BBB can be reconstructed by differentiation of hiPSCs into astrocytes, pericytes, and endothelial cells with subsequent co-culturing and encapsulation in hydrogel. In this induced BBB (iBBB) co-culture, brain endothelial cells form blood vessel-like structures, and pericytes migrate towards these vessel structures, highlighting the power of cell-cell interactions to facilitate assembly of cells into a functional in vitro model that recapitulates some in vivo cellular behaviors. When compared to APOE3/3, APOE4/4 hiPSC-derived iBBB co-culture demonstrated greater amyloid accumulation. To study the cell type-specific effects of APOE4, brain endothelial cells, pericytes, and astrocytes were differentiated from isogenic APOE3/3
and APOE4/4 hiPSC lines, and subsequently co-cultured in different genotypic combinations. Any co-culture that contained APOE4/4 pericytes, regardless of the genotype of brain endothelial cells and astrocytes, accumulated higher amyloid than co-cultures with APOE3/3 pericytes, indicating a critical role for pericytes in this process.

The “Brain on a Chip” in vitro model, developed to increase the complexity of the in vitro iBBB model, enables co-culturing of astrocytes, pericytes, endothelial cells, neurons, MGLs, and oligodendrocytes. This more complex system requires custom engineered extracellular matrix (ECM) for successful culturing. The model recapitulates in vivo cellular behaviors, including robust pericyte calcium responses to vasoconstrictor exposure and the formation of neurovascular units. The MGLs in this culture system are responsive to neuronal secreted factors as revealed by Ca++ imaging. APOE4 MGLs accumulate lipid droplets and are weakly responsive to neuronal cues. Moreover, APOE4 MGLs disrupt the activity of neuronal ensembles in the neuron-glia co-culture system.

3D brain-like tissue cultures from patient-derived iPSCs develop Alzheimer’s disease-related phenotypes
Giuseppina Tesco, MD, PhD, Tufts University

Seeding neuroprecursor cells (NPCs) in bioengineered silk-collagen scaffolds facilitates the influx of nutrients for long-term co-culture of neurons and astrocytes. To generate an in vitro model of AD, Dr. Tesco used this scaffold system to co-culture three-dimensional iPSCs carrying an amyloid precursor protein (APP) mutation that causes FAD; this co-culture system recapitulates some phenotypes observed in AD patients, including extracellular deposition of Aβ42 and increased Aβ42/40 ratio as well as increased neuronal activity.

Differential expression and gene set analysis in two-month-old co-cultures derived from control and FAD carriers revealed increased expression of genes involved in cytokine function, activated MGLs, and the neuronal cytoskeleton, along with reduced expression of genes involved in neurotransmitter regulation, neuronal connectivity, axon and dendrite structure, vesicle trafficking, and tissue integrity. At 4.5 months, fewer differentially expressed genes (DEGs) were detected, with cytokine genes being upregulated and genes involved in various neuronal functions being downregulated. Expression profiles of FAD carrier co-cultures were compared to 30 AD gene modules previously identified from over 1,200 human post-mortem brains. Two-month-old FAD cultures recapitulated human brain neurodegeneration transcriptomic signatures in one immune system gene module and four neuronal system AD modules.

Remaining challenges for the silk-collagen scaffold co-culture model include:

- Further optimization of this scaffold model and inclusion of additional cell types, including MGLs.
- Development of longer-term culturing protocols to model LOAD.

However, the model also offers additional research opportunities, including:
• Studies of well-characterized patient-derived iPSC and isogenic lines to study the impact of LOAD genetic variants in vitro.
• Further validation of the co-culture model using large datasets of human brain transcriptomic profiles.

Roundtable Discussion with Speakers and Participants

Recapitulation of In Vivo Systems
A common concern in using in vitro models is whether observed phenotypes are comparable to those observed in vivo or instead are artifacts of the iPSC system. In the context of AD, in vitro phenotypes cannot always be validated through comparisons to mice because of the significant differences in AD pathology between mice and humans. Recent single-cell RNA-sequencing (scRNA-seq) data has indicated that transcriptional differences between mouse and human brains are attributable mainly to non-neuronal cells, so validation of in vitro findings through mice may require the use of humanized models. The advent of scRNA-seq also provides researchers working with in vitro models access to a breadth of transcriptional data from the human brain, although data from post-mortem tissue should be interpreted with caution. In vitro model cells that are transplanted into mice for further study may better recapitulate some in vivo characteristics. Transplantation of hIPSCs into mouse brain can be used to investigate how human cells impact complex phenotypes in the mouse brain is an alternate approach that should be considered.

Recapitulation is also a major challenge to addressing the role of MGL in neuroinflammation. In vitro MGLs, including iMGLs in 2D and 3D models, do not recapitulate the properties of MGL in vivo. Technological improvements in the generation of more in vivo-like MGLs would thus significantly advance the utility of in vitro AD models for addressing AD pathogenesis.

More broadly, all disease models have limitations. Mice do not recapitulate all human AD phases and phenotypes, whereas in vitro AD models can be engineered for this recapitulation. One way to move the in vitro AD modeling field forward would be to establish a coordinated effort, similar to the Model Organism Development and Evaluation for Late-Onset Alzheimer’s Disease (MODEL-AD) Consortium, to study various hiPSC AD models while avoiding duplication of efforts.

Using hiPSC AD Models to Identify Useful Biomarkers
hiPSC AD models enable scalable efforts for AD biomarker discovery. New AD biomarkers can provide new opportunities for diagnostic tool and therapeutic development and can be used to assess whether a specific drug ameliorates a certain AD phenotype. However, it is necessary to distinguish between causal and correlational biomarkers, because correlational biomarkers can only be used as diagnostics, not drug targets.

Integration of Multiscale Model Data
The integration of data derived from multiscale biological models, including iPSCs, and in silico models of AD into a more cohesive dataset for data exploration may lead to additional insights.
into the pathogenesis of AD but will require development of additional data infrastructure and data sharing resources.

**Development of iPSC resources to address Alzheimer’s and Related Dementias**  
*Mark Cookson, PhD, NIA Intramural Research Program*

Gene variants associated with AD differ in allele frequency and effect size. Rare alleles with high effect sizes typically cause Mendelian disease, while common variants with lower effect sizes are associated with sporadic dementia; Dr. Cookson presented intramural NIA resources that use different research strategies to support identification of these two types of alleles.

**iPSC Neurodegenerative Disease Initiative**  
The iNDI project, which is an NIA-NINDS joint venture, aims to generate cell lines to enable further study of rare alleles. Although studying familial mutations that underlie early-onset FAD can provide insight into AD pathology and mechanisms, FAD is rare and so are FAD hiPSC lines. Therefore, as part of the iNDI initiative, NIH researchers have generated isogenic hiPSC lines in allelic series corresponding to rare alleles associated with AD, including knockout, tagged, and revertant hiPSC lines. The iNDI team selected parental hiPSC lines that contained neutral genetic risk for AD, were reliably editable, and could be readily differentiated into different neural cell types. Engineered hiPSC lines are now being characterized by transcriptomic and proteomic profiling and basic cellular microscopy. Lines have been distributed by iNDI to laboratories in the United States, Australia, and the United Kingdom and will soon be distributed by Jackson Laboratory.

**In Vitro eQTL Analysis**  
To understand more complex genetic variation, particularly in noncoding variants identified by GWAS for LOAD, NIA conducts eQTL analyses *in vitro*. eQTL analyses in hiPSCs enable more in-depth study of the transcriptome, proteome, and epigenome of key cell types, such as neurons and MGLs, that would otherwise be impossible in an *in vivo* model. Comparison of scRNAseq data between hiPSC lines and human brain tissue helps validate the *in vitro* model. This approach can also be used to model effects of genetic variation at a large scale. Once the effect of an eQTL is identified, the *in vitro* model can be used to test therapeutics as well as to screen for enhancer regions using CRISPR. This overall *in vitro* eQTL analysis paradigm is also scalable for study of large numbers of hiPSC donors.

NIA’s population scale iPSC study involves collecting PBMCs from the BLSA and GESTALT cohorts of healthy aging across the lifespan and reprogramming them to hiPSCs. Notably, current evidence indicates that an aging signature in the methylation clock is not preserved in hiPSCs. To analyze eQTLs at a population-scale, multiple hiPSC lines are pooled and differentiated into different neuronal cell types that are then subjected to scRNA-seq and single-cell Assay for Transposase-Accessible Chromatin (ATAC)-sequencing. The pooled data is deconvoluted and eQTLs and chromatin accessibility QTLs are catalogued for both iMGLs and induced forebrain-type cortical neurons (iFBNs). Subsequent downstream analysis will determine the relative effect size of the detected eQTLs.
Using stem cells to explore the genetics underlying brain disease

*Kris*ten Brennand, PhD, *Yale School of Medicine*

Neurodegenerative diseases are highly polygenic and highly heritable, although much of the heritability cannot be explained using common variants identified in GWAS analysis. Combinations of genetic variants can convey greater risk for developing a neurodegenerative disease, and single nucleotide polymorphisms (SNPs) can occur within eQTLs in both coding regions and non-coding regions. These SNP eQTLs can affect expression of a proximal target gene, and the aggregate of genetic risk for neurodegenerative disease is often convergent at the gene and biological pathway levels. Studying the interactions and synergisms of multiple risk alleles within eQTLs using stem cells enables robust data collection for complex computational analysis that can reveal causal variants.

GWAS analyses help identify variants in genes associated with increased disease risk, but prioritizing genes for further study of causality is challenging. A large GWAS of schizophrenia patients identified only one single SNP eQTL, out of 145 loci peaks, that was associated with schizophrenia and that affected expression of a proximal target gene, *FURIN*. Other GWAS loci contained dozens or hundreds of SNPs that were potentially associated with schizophrenia risk and that affected the expression of a nearest neighbor target gene. When a single putative causal SNP is resolved, Dr. Brennand’s research group uses CRISPR/Cas9 to engineer SNPs *in vitro* to determine whether the SNP affects expression of proximal or distant genes and cellular function.

To begin to investigate the polygenicity of schizophrenia risk, CRISPRa and CRISPRi are used in a combinatorial approach to manipulate the expression levels of multiple genes targeted by eQTLs associated with a disease. The interactions of the effects on the disease across multiple genes are elucidated by transcriptome analysis to determine whether transcriptomic changes are additive or synergistic. Notably, synergistic effects occurred between genes involved in the same pathway or closely related pathways and increased along with number of genes. Synergistic effects that increase disease risk tend to be stronger with the accumulation of multiple genes in the same pathway (e.g., synaptic, regulatory). Moving forward, a similar strategy will be employed to manipulate causal genes of AD originally predicted from post-mortem brain data in a cell type specific manner and potentially identify therapeutics that can ameliorate causal expression changes in those genes.

Modeling Alzheimer’s disease as a genetically heterogenous innate immune disorder

*Rudolph* Tanzi, PhD, *Harvard Medical School*

*In vitro* models have a number of advantages for studying AD, especially because mouse AD models do not recapitulate the same AD phenotypes seen in humans; for example, they lack neurofibrillary tangle (NFT) formation in response to β-amyloid deposition. Different *in vitro* models can be engineered and optimized to model different pathogenic cascades and phases of AD and then used to assess genetic mutations and to screen compound libraries for drugs that affect specific pathogenic mechanisms or phases of the disease.
3D Human Neural Cell Culture Model of AD

Initial in vitro studies using a 3D human neural cell culture model of AD containing neurons, astrocytes, and oligodendrocytes, but not MGLs, first showed that Aβ deposition directly induces tau pathology. hESC-derived neurons were transfected with APP/presenilin 1 (PSEN1) FAD lentivirus to stimulate production of Aβ oligomers. Over the course of four to six weeks in culture, Aβ plaques were detected, followed by the formation of NFTs.

Further investigation showed that hESC-derived neuronal cultures were capable of producing NFTs because these relatively mature cells produced an equal ratio of 3R and 4R forms of tau, both of which are necessary for NFT formation, whereas hiPSC-derived neurons produced only 3R tau.

3D Non-Cell-Autonomous Models of AD

To better understand the mechanism of NFT formation, a 3D non-cell-autonomous model of AD was used to expose naïve human NPCs (hNPCs) in 3D culture to exogenous Aβ oligomers produced by a 2-dimensional (2D) feeder layer of Aβ-producing hESC-derived neurons. After exposure to Aβ, the naïve hNPCs generated NFTs from endogenous tau. Mutations in Aβ-producing cells that increased the Aβ42/40 ratio also led to higher levels of Aβ oligomers, Ptau, and NFTs, showing that NFTs depend on the formation of Aβ oligomers, which is driven by the Aβ42/40 ratio. In addition, increasing the Aβ42/40 ratio in iPSC-derived human cortical organoids was sufficient to drive an increase in Ptau, NFT, and cell death. Integrated pathogenic pathway analysis revealed that 3D AD models with high levels of Aβ42 most closely recapitulate the human AD brain.

3D Triculture Model of AD

The 3D models described thus far recapitulate multiple pathogenic pathways observed during the early (presymptomatic) proteostasis phase in human AD brains, including transcriptomic changes and Aβ pathology that drives tauopathy. However, other 3D models are required to understand how AD pathologies drive neuroinflammation, which leads to the degree of synapse loss and neuronal cell death required for cognitive impairment. Recent AD GWAS have identified candidate genes for further functional studies that are associated with innate immunity, MGLs, and neuroinflammation. The first of these genes, CD33, was shown to be associated with AD in a family-based GWAS published by Dr. Tanzi and colleagues in 2008 (Bertram L et al. 2008 Am. J. Hum. Genet.), followed by TREM2 several years later. Changes in the expression of some genes involved in microglial innate immunity regulate whether MGLs remain in the housekeeping pathway and clear Aβ or switch to the inflammatory pathway that leads to neuroinflammation as well as excessive synaptic pruning and reactive oxygen species production. To address the role of MGL activity in AD pathogenesis, researchers used a 3D triculture model to house the original 3D co-culture of neurons and astrocytes in a core culture region surrounded by MGLs, which can be attracted to pass through microfluidic channels to engage with the core. In tricultures of wild-type neurons and astrocytes, MGLs did not pass through the channels to engage with the neuron-astrocyte core. However, neurons that produced Aβ plaques and NFTs triggered enrichment of MGLs in the neuron-astrocyte core, which led to synaptic pruning, neuronal damage, astrogliosis, increased phospho-tau formation,
and cell death. This 3D triculture model was scaled to a 96-well plate format with vertical channels for MGL entry, making the model amenable to genetic screens using CRISPR/Cas9 and drug screens.

This *in vitro* system can also be used to compare the activity of iMGLs to *in vivo* MGLs. Notably, in the 3D triculture model, iMGL activation increased tauopathy compared to *in vitro* models without MGLs. Transplantation of wild-type and AD iMGLs into mouse brain enabled comparison of transcriptional responses to transcriptional responses in the 3D tri-culture model. scRNA-seq data indicates very similar transcriptional responses between the *in vivo* and *in vitro* models. Moving forward, CRISPR/Cas9 can be used to knock out genes in iMGLs to study gene functions.

**Involvement of Peripheral Immune Cells in AD Pathology**

To study peripheral immune cell contributions to AD pathology, a modified vertical triculture model was developed to include horizontal channels that allow peripheral immune cell passage. The infiltration of T-cells into AD neuron-astrocyte core cultures was higher than into wild-type core cultures. This increased infiltration is independent of the presence of iMGLs, but the presence of both T-cells and iMGLs leads to more cellular damage compared to either T-cells or iMGLs alone. In contrast, while monocytes also exhibit increased infiltration in AD cultures compared to controls, the monocytes do so independently of the presence of iMGLs.

**3D BBB Model of AD**

The role of BBB breakdown in AD pathogenesis can also be studied using *in vitro* models. The original monolayer BBB model recapitulates several key aspects of BBB dysfunction observed in AD patients, including increased permeability, decreased expression of adhesion proteins, increased reactive oxygen species production, and deposition of Aβ peptides at the vascular endothelium, showing that AD pathology can contribute to BBB breakdown in this model. A newer 3D-AD BBB model includes a microvascular network achieved by co-culturing hiPSC-derived endothelial cells, pericytes, and astrocytes. In response to AD pathology, the vasculature in this model exhibited hypervascularity, decreased vessel diameter, and increased dead-end sprouts.

**Feasibility of Drug Screening in 3D AD Models**

The 3D AD models described by Dr. Tanzi successfully modeled the following observations from human brains: Aβ oligomer induction of NFTs, dysregulation of specific signaling pathways, neuroinflammation, MGL activation, peripheral immune cell infiltration, and BBB dysfunction. These *in vitro* models can serve as cheaper, more feasible vehicles for drug screening compared to more expensive and time-consuming mouse models and less permeable organoids. The use of *in vitro* models raises the possibility that repurposed drugs and natural products identified in screens could bypass preclinical studies in mice and proceed directly into Phase I trials. For example, a 3D AD model that recapitulates Aβ42/40 AD pathology was used to identify 38 drugs and 43 natural products that selectively reduced tau pathology independently of effects on Aβ. The model was also used to show that γ-secretase modulators, which reduce the Aβ42: Aβ40 ratio, attenuated P-tau and NFTs without inhibiting γ-secretase activity on its other substrates,
e.g., Notch. The most promising γ-secretase modulator is entering Phase I trials in late 2022/early 2023. Dr. Tanzi’s research group also identified over 50 known drugs and natural products that induce microglial uptake and clearance of Aβ, which could be tested as cheaper and safer alternatives to Aβ immunotherapies. Dr. Tanzi also used the 3D AD model to identify over 60 known drugs and natural products that reduced microglial release of pro-inflammatory cytokines in response to activation with bacterial lipopolysaccharide.

Roundtable Discussion with Speakers and Participants

Importance of In Vitro AD Models in Drug Development
Accelerating AD drug development may require adjustment of the typical drug development pipeline. Mouse models do not recapitulate all human AD pathologies, and 3D in vitro AD models may be better suited for drug discovery tasks. More importantly, mouse models do not capture the array of genetic variation that underlies risk and resilience to AD. Some discussion participants suggested that if a repurposed drug or natural product is found to ameliorate an AD phenotype in vitro, studies of that drug may be able to bypass preclinical mouse studies and proceed directly to higher order organisms (e.g., non-human primates) or clinical trials.

Study of Individual Variants Versus Common Pathways
Polygenic disease risk and mechanisms should be studied through common pathways rather than individual variants due to disease complexity and interaction of multiple risk alleles. Some participants suggested that the future of AD treatments will likely focus more on disrupted pathways than symptoms. In addition, studying AD in terms of disrupted pathways can provide multiple druggable targets across multiple disease phases. For example, a compound that clears Aβ plaques would serve better as AD prevention than as treatment because plaques form well in advance of downstream AD neuropathology. Compounds that can reduce neuroinflammation may be effective in treating AD after disease onset because neuroinflammation occurs later in the disease process.

Modeling Environmental and Lifestyle Factors In Vitro
Environmental and lifestyle factors contribute to brain health as well as the risk for developing AD. Strategies for controlled in vitro modeling of environmental and lifestyle factors associated neuroinflammation can help determine the cellular and molecular mechanisms that underlie these effects. For example, induction of neuronal injury in vitro could be used to model brain injuries that increase neurodegeneration risks. In vitro AD models could also be used to model exposure to environmental toxins that humans typically encounter.

One of the main lifestyle interventions that can improve brain health is sleep. During sleep cycles, MGLs clear brain debris, including Aβ and amyloid, and sleep disturbance is associated with AD. Improving methods to culture hiPSC-derived MGLs would provide an opportunity to investigate the role of MGL in Aβ and amyloid clearance during sleep and to identify small molecules that can stimulate this process. Similarly, the mechanisms through which other beneficial lifestyle factors, such as exercise and the gut microbiome, reduce neuroinflammation and activate MGL-mediated plaque clearance could also be studied in vitro.
**Modeling Aging Using hiPSCs**

Most *in vitro* models are currently not positioned to effectively model human aging and integrating aging into these models is a critical challenge. One major challenge is understanding the extent to which cells derived from hiPSCs retain the cellular and molecular hallmarks of aging. The large number of human brain aging datasets provides an opportunity to calibrate and validate *in vitro* models of healthy aging. Modeling brain aging *in vitro* also requires the field to develop a comprehensive definition of the mechanisms that mediate aging; *in vitro* experimentalists could then model different aspects of this definition. Different aspects of aging that could be combined to formulate a formal definition include: (1) changes in the epigenome, including the methylation clock, (2) increased cellular senescence, (3) oxidative damage, (4) disrupted proteostasis, (5) shifts in metabolic pathways, (6) inflammation, (7) mitochondrial dysfunction and impaired bioenergetics, (8) transcriptional changes, and (9) spread of neuropathologies. Aging can also be viewed through the lens of different kinetic and stoichiometric changes that eventually desynchronize critical molecular actions.

Possible approaches for modeling aging include recapitulating cell niches that can drive cellular aging or exposing cell cultures to certain paracrine factors. Based on transcriptional changes observed in human brain datasets, researchers could identify causal transcriptional changes capable of inducing cellular aging in culture. However, an important consideration is that different cell types may age at different rates and through different mechanisms. Because *in vitro* co-culture systems enable more precise control than mouse models over the genetic backgrounds of different cell types, introducing different combinations of non-coding mutations into different cell types within the same co-culture system could provide a more fine-tuned aging model that captures human heterogeneity. Directly converted neurons are a useful aging model because they retain aging hallmarks; however, current protocols for direct conversion are complicated, and post-conversion, these neurons cannot survive shipments to other laboratories.

When developing strategies to model aging *in vitro*, researchers should consider the differences between the processes of aging and maturation. *In vivo* models have shown that cell maturation often relies on specific cues from the microenvironment. Identifying niche and paracrine signaling cues and integrating them into *in vitro* co-culture models may improve *in vitro* recapitulation of *in vivo* cellular maturation of different cell types. The process of aging requires additional inputs, and modeling common stresses *in vitro* may help model these inputs. More complex triculture models can also help elucidate what secreted factors attract beneficial peripheral immune cells, such as monocytes, that can curtail AD pathology versus harmful immune cells, such as T cells, that exacerbate AD pathology.

**Conducting hiPSC Research at a Larger Scale**

Studies of more complex diseases, such as AD, require research at a large scale. Rather than focusing on a single gene at a time, researchers need to develop new strategies for multi-omics and pooled screening approaches. Understanding what combinations of variants drive different AD phenotypes may require machine learning and more complicated analyses, which would require collaboration with computational researchers.
Improving Communication Between Computational and Experimental Researchers

Combining *in vitro* AD modeling with computational modeling can yield new disease insights. Experimentalists have generated many large datasets but are currently unable to effectively integrate multiple data streams. Improved communication between experimentalists and computational researchers can help drive effective data integration and subsequent analyses. Computational researchers could also use AI tools to identify candidate compounds that affect AD pathologies, and experimentalists can test those compounds *in vitro*. To promote these types of collaborations, NIH could consider a funding mechanism that requires formal collaboration between experimentalists and computational researchers.

Session II: Exploring the Challenges and Potential Value of Modeling Basic Mechanisms of Brain Aging and AD *In Silico*

*Session Chair: William Lytton, MD, SUNY Downstate Health Sciences University*

Dr. William Lytton provided an introduction to *in silico* modeling and simulation in the brain, highlighting three examples to illustrate the wide range of ideas and approaches to modeling brain function and dysfunction. Brain theory is a classical physics or mathematical modeling approach, which entails dimensional or analytic reduction to model brain function from a theoretical perspective. Artificial neural networks (ANNs) and deep learning approaches can be applied in two ways. First, because ANNs derive information from the brain, they can be used as a model of the brain. A second and widely used application is the artificial intelligence (AI)/machine learning usage of ANNs as highly dimensional highly nonlinear statistical techniques. Multiscale modeling (MSM) entails highly complex simulations that allow an understanding of how changes occurring at one level of simulation can propagate to higher levels. Session II presentations and discussions addressed the challenges, opportunities, and value of *in silico* modeling of brain aging and AD.

Multiscale modeling of dementias, from molecules to Stroop

*William Lytton, MD, SUNY Downstate Health Sciences University*

MSM attempts to solve the complexities of the brain to provide an integrated description of the interplay across spatial and temporal scales of neural organization. One difference between MSM of the brain compared to other organs is that whereas MSM of other organs cleanly separates different scales (e.g., cell versus local tissue organization), MSM of the brain entails some overlap between scales. For example, neuronal dendrites can reach across many layers of cortex and therefore interact in complex ways with the network itself; these overlaps mean that neurons cannot be cleanly encapsulated as a cell with a particular identity. The MSM perspective is distinct from artificial neural network or simplified point neuron modeling approaches. MSM is proving useful for modeling complex neurological disorders such as AD and other dementias (e.g., disentangling how risks and causes combine in complex systems to produce disease), and addressing how these diseases might be prevented or ameliorated by combinations of multi-staged, multi-target pharmacological and nonpharmacological therapeutic interventions.
One emerging use of MSM is in digital twins. Digital twins are virtual representations designed to accurately reflect a physical object or system; these twins can use simulation, machine learning, and reasoning in combination to generate mechanistic insights that can be applied back to the physical object. The goal of building these models in neuroscience is to retain as much complexity as is needed to enable evaluation of which factors (e.g., microglia, neuroinflammation) are important in specific aspects of brain function and dysfunction. Initial efforts are underway to build a digital twin model of neocortex using large-scale, AI-segmented electron micrographs based on the IARPA Microns Dataset of mouse cortex and the H01 Dataset of human cortex. However, building models using these datasets is challenging because of the complexity of neuronal morphology and differences between current database structure and the data structure required for model simulations. This example highlights both the opportunities and challenges of obtaining experimental data for use in developing brain models and simulations. Ongoing and future research avenues for digital twin projects include application to cognitive tasks, simulation of amyloid and tau diffusion in tissue, synaptic scaling in AD progression, and circuitry changes in AD.

**Redundancy and realism in biological vs artificial neural networks**
*Cian O’Donnell, PhD, Ulster University*

**Neural Circuit Function Redundancy in Brain Disorders**
Although recent evidence shows that up to one hundred genetic mutations are correlated with risk for complex brain disorders, the clustering of cognitive and behavioral symptoms, which form the basis of specific disease diagnoses, implies points of phenotypic convergence within levels of organization in the nervous system. Convergence might happen at the level of molecules, cells, circuits, or cognition and behavior—or at a mix of levels. These possibilities highlight the need for *in vitro* and *in silico* research to identify, understand, and therapeutically target the points of convergence.

Convergence is related to the ubiquitous property of redundancy in the nervous system and to two of its consequences, sloppiness and dependencies. In the context of brain circuitry, redundancy means that very different configurations of cellular and synaptic components can enable the same neural circuit functions. Sloppiness is the idea that high-level brain circuit properties are not equally sensitive to changes in different parameters, such that perturbations of different parameters can differentially impact overall circuit function. Co-tuning of multiple circuit parts during development can lead to strong dependencies between their effects on overall function. Although these concepts were pioneered and have been investigated empirically and computationally for the past 20 years through the work of Eve Marder and colleagues on the crab and lobster stomatogastric ganglia, their implications for understanding and treating brain disorders are not yet clear. Potential implications include:

- Redundancy might mask the effects of distinct mutations that are not observable in higher level cognitive function, but that confer differential responses to treatment.
- Sloppiness implies that not all biological disease effects matter and that some are benign with respect to circuit function.
• Designing therapeutic interventions that target biological disease components in isolation without account for dependencies among biological components that co-vary could exacerbate circuit-function symptoms.

**Deep Neural Networks as Models of Brain Function and Dysfunction**

Deep neural networks (DNNs) provide another approach to modeling brain function and dysfunction. However, although DNNs are similar in structure to the brain, with components that look like neurons connected to each other in input and output layers, they are very simplified compared to real biology, and phenomena such as intracellular signaling, ion channel dynamics, cell types, neuromodulation, development, and evolution do not exist. A key consideration when using DNNs to model brain functions such as neural computation and learning is thus which physiological details matter and which do not.

The primary reason DNNs are used as models of the brain is that they are consistent with the dominant view that brain computations involve the coordinated electrical dynamics of populations of neurons across multiple brain regions. However, while representations by DNNs and the brain appear to be very similar for some measures (e.g., the visual system), current DNNs notably miss potential computations performed by molecular signaling. From a brain learning perspective, DNNs are trained using supervised learning (i.e., the process of making an algorithm learn to map an input to a particular output), a learning paradigm that is biologically implausible and is inconsistent with the dominant learning paradigms in neuroscience: 1) unsupervised activity-dependent plasticity (e.g., Hebbian learning), and 2) reinforcement learning (e.g., neuromodulation by dopamine). Thus, DNNs are brain-like in some ways and can be trained to do complex tasks, unlike classic models in computational neuroscience. However, many biological mechanisms are not incorporated into DNNs, which may result in representational properties and computational strategies that create misleading predictions about brain function.

**Multiscale computational modelling in brain ageing and disease: from intracellular networks to cell-cell interactions in the tissue**

*Antonio del Sol, PhD, University of Luxembourg*

Application of MSM approaches to stem cell biology and regenerative medicine and to brain aging and disease can shed light on biological mechanisms of these processes and guide the development of novel therapeutic strategies. MSM that spans different levels of biological organization (e.g., intracellular, cell-niche, cell-cell, and inter-organ interactions) and relies on experimental data to generate predictions that can be experimentally validated *in vitro* or *in vivo* illustrates the power of this approach and highlights the synergy between experimental and computational efforts.

One key challenge in stem cell research that MSM can address is how to rejuvenate stem cells in aged tissues. MSM was used to model the niche (i.e., environmental) effect on stem cells in order to identify cues from the young niche that maintain stem cells in a particular phenotype, with the goal of counteracting the detrimental cues from the old niche as a strategy for cell
rejuvenation. A computational tool, SigHotSpotter, was developed to identify high probability stem cell signaling molecules that mediate the signal from the niche to transcription factors in the stem cell gene regulatory network. This method identified a signaling molecule, Sfrp5, that maintains neural stem cells (NSCs) from old mice in a quiescent state and that, when blocked, increases the activation of NSCs as well as neurogenesis in old mice.

To model dysregulated immune responses, such as chronic inflammation in neurodegenerative diseases, a single cell-based MSM computational method called InterCom was developed to analyze cell-cell communication for the systematic prediction of protein targets to modulate the inflammatory response. This tool was then used to predict immunomodulatory target proteins that underlie hyperinflammatory responses in patients with COVID-19, which were validated experimentally. These findings suggest that this computational methodology can be leveraged to systematically identify therapeutic targets for modulating hyperinflammation in infectious and chronic diseases.

Challenges and opportunities for MSM of brain aging and disease include:

- Identifying appropriate in vitro and in vivo systems for modeling dysregulation of biological processes associated with brain aging and disease and for testing potential therapeutic strategies to revert the aging and disease phenotype
- Leveraging and integrating emerging omics data (e.g., spatial transcriptomics, single-cell phosphoproteomics) to develop more accurate computational models
- Using multiscale analysis and modeling to enable a more holistic view of the mechanisms underlying brain aging and disease phenotypes
- Applying computational biology to guide the design of novel therapeutic approaches to treat aging-related neurodegenerative diseases (e.g., cell therapy, drug repurposing).

**An in silico lens into hippocampal learning and memory**

*Yuri Dabaghian, PhD, University of Texas Health Science Center at Houston*

One goal of in silico modeling of AD and aging phenomena is to build a comprehensive “functional stairway” that links neural mechanisms from cells to circuits to networks to behavior and cognition. While many studies have addressed cellular and cognitive mechanisms of AD, far fewer have focused on mechanisms at the circuit, synchronization, and ensemble levels. This gap may stem from the difficulty of explaining emergent phenomena such as brain waves and synchronized oscillations based on the properties of individual cells, or cognitive phenomena based on the properties of neuronal spikes. Integrating large volumes of information to understand such emergent phenomena requires both a conceptual framework and substantial in silico computational effort.

Spatial learning and memory is a primary cognitive target of AD and the hippocampus plays a central role in spatial cognition by creating an internal representation of space, known as a cognitive map. Thus, one way to understand cognitive decline in AD and aging is to understand how cognitive maps in the hippocampus deteriorate. Experiments in which hippocampal CA1 place cell spiking activity was recorded in rats as they ran on a shape-changing track showed
that place field maps are flexible and suggested that the sequence of place cell firing does not depend on the geometry of the environment, but rather the sequence in which places (i.e., memory elements) appear.

From a mathematical perspective, this sequence template can be viewed as a topological map, which is a constructive object that can be built and studied using mathematical and computational tools to understand the contributions of individual elemental structures to the topological map. Dr. Dabaghian and colleagues used this approach to develop a computational framework in which the elemental structures of the hippocampus are viewed as neuronal cell assemblies in CA3 and downstream readout neurons in CA1. This simple and well-defined two-layer construction allows modeling of the effects of synaptic connections on the spiking parameters of CA1 neurons, how spike train parameters produce synchronized activity (i.e., brain waves) within the network, and how the hippocampal topological map of space emerges from combining all of this information. This network model can be used to address how different parameters of neuronal and network activity (e.g., firing rates, place cell sizes, number of cells) influence neuronal assembly and topological map outputs in normal hippocampal function and how changes in these parameters lead to problems with constructing topographical maps of space and poor spatial cognition in AD. For example, the model was able to show that the time required to build the topological map increases as synapses weaken, as occurs in AD. In another example, in silico suppression of brain waves showed that spike modulation by brain waves is essential for successful space coding; this perspective can be used to interpret changes in synchronicity observed in AD brains and why changes in specific parameters are detrimental for learning and memory. Future studies will use this model to study hippocampo-cortical interactions and how they are impaired in aging and AD.

FAIR resources and infrastructure for AD target discovery and validation

Anna Greenwood, PhD, Sage Bionetworks

A better understanding of the complex biology and heterogeneity of AD presentation and risk is critically needed to diversify and improve the therapeutic pipeline. NIA has funded a series of initiatives across that pipeline, including multiple research consortia to generate insights into the biology of AD (e.g., AMP AD, M2OVE-AD, Resilience-AD). These programs feed their data and insights into several downstream consortia (e.g., TREAT-AD, MODEL-AD) that generate resources to accelerate the study of proposed targets (e.g., target enabling resources, next generation animal models). Data sharing infrastructure is essential for generating, accessing, and utilizing the wealth of data being generated through these initiatives, which embrace open-science principles and share all data and outputs with the broader research community. Sage Bionetworks, the data management core for several of these consortia, has built openly accessible data sharing infrastructure to distribute the data tools and research resources emerging from these programs. Resources likely to be of particular interest to workshop participants include the AD Knowledge Portal, Agora, and the MODEL-AD Explorer. The AD Knowledge Portal, for example, is a catalogue that allows exploration of raw data, results, and tools generated by the NIH initiatives to support discoverability and provide data context. The wide range of data and results includes diverse specimens (e.g., human, mouse, hiPSCs), data
types (e.g., multi-omics, behavior, imaging, electrophysiology), and levels of processing (e.g., raw, processed, analytical outputs), which have been curated and harmonized across studies.

To enable the community to better leverage these kinds of data, Sage Bionetworks is pursuing an opportunity to apply feedback from the later stages of the drug development pipeline back into earlier stages. In particular, computational teams (e.g., AMP-AD) who are developing methods for target discovery and prioritization would benefit from incorporating experimental results (e.g., iPSC data) to better validate their models. However, substantial challenges exist around data availability, curation, harmonization, and machine readability. The MODEL-AD Explorer, which facilitates evaluation of phenotypic data from mouse models developed by the MODEL-AD consortium, provides a useful framework for expansion to share a growing set of experimental validation data. This or similar infrastructure can ultimately be used for other model systems, along with data pre-processing and harmonization pipelines, to develop a results explorer for AD validation studies that provides access to curated experimental results, including a standard set of omic and phenotypic data from other model systems, including iPSC models. Although developing this type of resource will be challenging, it also presents numerous opportunities for making the valuable data from experimental studies more findable, accessible, interoperable, and usable.

**Roundtable Discussion with Speakers and Participants**

*Sharing of Computational Modeling Tools*
Sharing computational models and modeling tools is essential for enabling use by experimentalists and other computational researchers and for ensuring the reproducibility of modeling results. To facilitate this sharing, Sage Bionetworks catalogues and provides links to modeling tools within the AD Knowledge Portal. Specific tools that are appropriate for explorers (e.g., Agora or MODEL-AD) may be incorporated into the platform. Some groups of computational researchers actively promote model sharing through other forums. Individual workshop speakers expressed a general willingness to share their modeling scripts and tools, although successful implementation of some tools might require collaboration to appropriately organize and link scripts.

*Challenges and Opportunities with Incorporating iPSC Model Data into Explorers*
Building a data sharing infrastructure for experimental data generated by in vitro model systems presents a distinct set of challenges from those faced by the MODEL-AD Explorer. Whereas MODEL-AD provides access to multiple phenotypic readouts from individual mouse models, in vitro models of aging and AD utilize a wide range of both experimental approaches (e.g., organoids, gene knockouts, transgenics) and phenotypic readouts (e.g., high-throughput assays, individual cell counts). This lack of standardization presents significant challenges for developing a common data sharing infrastructure.

Efforts to build infrastructure could benefit from discussions between experimentalists and computational researchers about what data sharing strategies and approaches optimally bridge the needs of both communities and foster interaction and collaboration. For example, these
conversations could clarify how data need to be standardized and presented to render them most useful for modelers.

**Challenges for Applying MSM Tools to Brain Aging and AD**

Despite the availability and value of the different MSM approaches highlighted in the workshop, relatively few MSMs have addressed brain aging or AD. In contrast to psychiatric disorders, where MSM attempts to fill the wide gap between molecular and cellular properties and cognitive symptoms, applying MSM to AD offers the possibility of gaining insights into mechanisms and potential treatment approaches based on single cell properties. However, developing MSMs for AD has thus far been limited by uncertainties about the impacts of different levels and types of pathological hallmarks and mechanisms (e.g., amyloid, tau, inflammatory cells) on circuit function.

Closing the gap between the computational and experimental communities is also essential for promoting the development of MSMs that address aging and AD, but meeting this challenge requires sustained effort. Discussion participants suggested that experimentalists should engage with modelers during the conception and initial planning of research projects in order to define questions, generate suitable data and predictions, and determine limitations. The computational community can also engage with the experimental community to define the most relevant and valuable questions for the field. Participants suggested that funding mechanisms to support collaborations between experimental and computational researchers and initiatives that encourage computational researchers to attend experimental conferences would be particularly valuable for defining questions that modeling can address and for promoting collaborations to address those questions.

**In Vitro and In Silico Modeling of Aging**

Computational modeling of healthy aging, not only of AD, is critically important for experimentalists studying aging in *in vitro* systems. Building computational models that incorporate cellular level hallmarks of aging for different neural cell types could serve as an important guideline for experimentalists trying to recapitulate aging in a dish.

**Session III: Consideration of the Ethical Implications of Modeling Mechanisms of Brain Aging and AD In Vitro and In Silico**

*Session Chair: Matt Sutterer, PhD, Division of Neuroscience, National Institute on Aging*

**Modeling basic mechanisms of brain aging: re-invent the wheel of research ethics or add new spokes?**

*Insoo Hyun, PhD, Harvard Medical School*

Modeling mechanisms of brain aging and AD *in vitro* and *in silico* necessitates consideration of both immediate and future ethical challenges. Although some of the ethical complexities of *in vitro* and *in silico* modeling concern questions that the field has yet to address or even articulate, researchers can reinforce and expand existing structures in research ethics to prepare for these emerging challenges. Promising avenues to enhance the ethical foundation of
research include implementing future-oriented informed consent practices, attending to potential ethical implications of increasingly realistic in vitro models, and ensuring consistent and rigorous ethical oversight of preclinical studies.

When obtaining informed consent from cell line donors, researchers should communicate both the intended and foreseeable possible uses of specimens, including research in organoid models and hiPSCs. Using informed consent language that encompasses these possibilities ethically serves donors and preserves the utility of prospective and biobank specimens even as the rapid pace of scientific development transforms experimental methods. Researchers can inform donors that no biological evidence currently prompts concern related to consciousness or pain perception in organoid models; however, future advancements may raise more significant ethical challenges. Methods that increase the realism of organoid models to recapitulate human development (e.g., vascularization, linking of organoid models into assembloids) are needed to develop a preclinical evidence base that can ethically and scientifically support first-in-human clinical trials. These increasingly realistic brain organoid models may present corresponding increases in ethical complexity.

Contending with emerging ethical challenges in in vitro and in silico brain aging research requires rigorous oversight of both ethics and efficacy, which may necessitate augmentation of the current review structure for preclinical studies. Institutional review boards (IRBs) may misattribute the responsibility to consider ethics and efficacy in preclinical studies to surrogate review sources (e.g., investigational new drug authorization, funders, data and safety monitoring boards), resulting in insufficiently rigorous IRB analysis. In addition to reinforcing appropriate oversight of preclinical studies, researchers should consider how to fulfill the ethical obligation to ensure that in vitro and in silico studies generate reproducible findings that can be generalized to larger populations, which requires both sufficient power and genetic diversity.

The ethical dilemma of brain models

*Henry T. Greely, JD, Stanford University*

Significant ethical challenges may result from both potential failures and successes of in vitro and in silico models at accurately simulating brain aging. Although the current landscape of in vitro and in silico models does not raise major ethical concerns, researchers must pay attention to issues that could emerge related to data privacy and ownership, the realism of models, and the potential implications of the public interpretation of, and reaction to, research in brain models.

Collecting human cells and data to model brain aging in vitro and in silico requires obtaining donor consent for not only the use of cells in models, but also for their inclusion in broad sample or data sharing initiatives. Donors’ likely misunderstanding of the potential uses of their samples or data or of the limitations of deidentification could lead to unhappy or angry reactions if they learned of uses that troubled them. Such reactions could in turn result in reduced trust and a more negative public perception of research in general. To mitigate these potential conflicts, researchers should donors with realistic expectations of the possible uses of
their data and the risks of reidentification. Researchers must also consider whether donors have any ethical claim to the knowledge or profit gained through modeling performed using their cells—particularly in the case of \textit{in vitro} models using hiPSCs, which could lead to the discovery of patient-relevant health information (e.g., genetic mutations and disease risks) or the development of commercial products.

Sciences use models for the human brain in part because of legal and ethical limits on using living humans as subjects. As brain models become increasingly realistic, some of the ethical and legal limitations that apply to human and animal research may become relevant to their use. Although current \textit{in vitro} and \textit{in silico} models have yet to approach this threshold, the public, influenced by excited headlines, may overestimate the extent to which organoid models simulate cognitive function. This possibility argues for preemptive negotiation of these ethical concerns, particularly when media overstate scientific progress or use language that creates misleading impressions (e.g., calling organoids “mini brains”). Researchers should also consider the potential public reaction associated with testing approved drugs or natural substances in clinical trials based on results obtained using \textit{in vitro} models. These trials may ignite public demand for products before they have sufficiently demonstrated either safety or efficacy for brain aging uses, which also enables potential exploitation of that demand. Thus, researchers should use caution when proposing to move directly to clinical trials from \textit{in vitro} studies and, if possible, should identify intervening models to demonstrate efficacy. Researchers and funding agencies also bear ethical responsibility for allocating efforts and resources with careful consideration of the promise and potential impact of proposed research.

\textbf{Roundtable Discussion with Speakers and Participants}

\textit{Communication with Cell Donors}

Ethically procuring samples with the greatest potential for future use requires an informed consent process that includes dialogue with donors and their families about possible future research developments. Discussion participants emphasized that modeling that recapitulates early human life (e.g., embryo modeling) should use cell lines from donors who have explicitly consented to that use, which may require re-consent from prior donors or prospective collection for future research. Researchers can also consider how to communicate with donors and families about organoid models that recapitulate early development in order to discourage potential perception of a connection between the model and the donor. In addition, ethical complexities may arise when \textit{in vitro} models generated from healthy controls result in incidental findings that cannot be clinically validated.

\textit{Preclinical Models to Establish Efficacy}

Tension exists between the need to advance AD treatments that may alleviate suffering into clinical trials and the need for a robust preclinical evidence base that demonstrates the efficacy of potential AD treatments despite the shortcomings of available nonhuman models. Testing drugs and natural substances already approved for human use in 3D models could provide some evidence of safety or efficacy to support human clinical trials and bypass studies in rodent models. However, because 3D models are unlikely to predict perfectly effects in the full environment of a human brain, undertaking clinical trials that may encourage public use of
products prior to establishing safety or efficacy in better-validated models may raise ethical concerns.

Confirming that in vitro models offer the best available means of demonstrating efficacy in humans requires rigorous methods to validate these models beyond disease-specific contexts and to ensure researchers can accurately distinguish between significant and artifactual findings. Overestimating the accuracy of models may increase risk for individuals whose health relies on the data generated. Currently, researchers use transcriptomic data to benchmark 3D organoid models against adult human brains and must carefully consider whether the cells used in the model are appropriately matched to the specific processes targeted in the research.

**Reproducibility and Generalizability of In Vitro Models**
The number and variety of cell lines needed to ensure that the results of in vitro studies are both reproducible and generalizable depends on the research question under consideration. Generating edited cell lines and culturing them in identical environments reduces variables and enables researchers to identify meaningful patterns with fewer cell lines. Researchers use both discovery and replication data sets to assess whether differential responsiveness among cell lines corresponds to genomic differences and are working to expand the diversity of cell lines under study. However, obtaining iPSC lines that capture a representative sample of the global population remains a challenge.

**Determining Research and Funding Priorities**
Predicting which research offers the most impactful use of resources presents challenges for researchers, peer reviewers, and funders alike. Researchers face pressure to ensure continuation of funding, which may result in prioritizing incremental results that support grant renewal over promising ideas with higher risk. This is an ethical issue as well as one of science policy. At the level of funding structures, diversifying approaches and priorities may improve both short-term and long-term results in in vitro and in silico research modeling mechanisms of brain aging and AD.

**Session IV: Final Panel Discussion and Wrap-Up**
*Session Chair: Amanda DiBattista, PhD, Division of Neuroscience, National Institute on Aging*

**Discussion of Overall Research Gaps and Opportunities for Progress**

**Multiscale Modeling of Aging**
The scale for modeling aging in vitro depends on the research question and observed phenotype. Different hallmarks of aging are observed at different scales of brain organization and at different stages of the aging process. For example, DNA damage, epigenetic changes, and cellular senescence occur within individual cells, while changes in cell-cell and tissue-tissue interactions occur at a cellular network level. Participants suggested that the in vitro modeling community has an opportunity to define a set of metrics and biomarkers—which may or may not include disease pathology—to define and measure aging; these metrics may differ by cell type, because normal aging affects cell types, such as neurons, astrocytes, and MGLs,
differently. More integrated models of aging will require a multiscale, holistic view of multiple *in vitro* models.

**Addressing Current Limitations of In Vitro Models**

Disease modeling in *in vitro* systems has limitations that participants suggested should be considered when interpreting results and integrating both *in vitro* and *in vivo* data. Some *in vitro* disease modeling limitations specific to AD include: (1) lack of sequestration or clearance of mediators produced by MGLs, astrocytes, and other cells, which can cause stagnation of the cellular environment, and (2) presence of non-physiological or pathological phenotypes due to trans- or de-differentiation of cell types.

*In vitro* co-culture systems are still in development and require further optimization. Participants suggested that the implementation of perfusable systems within organoid cultures can improve their value as model systems. In addition, an influx/efflux system could be implemented to further mimic *in vivo* conditions. Incorporating peripheral cells and factors, such as nutrients and other molecules that enter the brain via cerebral blood flow, is also an important consideration.

**Collaboration Between Computational and Experimental Researchers**

Participants noted that research collaborations between experimentalists and computational researchers are mutually beneficial relationships. Computational researchers provide invaluable skills to manage and analyze large datasets, while experimentalists have the ability to generate large datasets useful for computational research projects. Forming collaborations before designing a project and elaborating experimental details can ensure useful data and correct metadata are collected. Computational researchers can also provide insight on strategies to use AI to benchmark particular *in vitro* models against human systems in order to improve recapitulation of *in vivo* phenotypes.

Participants suggested that the current status of overall communication between experimentalists and computational researchers may be impeding the formation of valuable, long-term collaborations. Computational researchers sometimes feel that experimentalists are less willing to listen and learn about computational models and ideas, while computational researchers need to understand the research projects of experimentalists in order to use their datasets to build useful models. Earlier career experimentalists may struggle to form collaborations with computational researchers; however, participants noted that structured opportunities and larger-scale research projects such as AMP AD have helped experimentalists network and collaborate with computational researchers.

**Direct Conversion of Neurons Versus hiPSC-derived Neurons**

Directly converted neurons preserve several aging phenotypes that are not found in hiPSC-derived neurons. These aging phenotypes include transcriptomic and epigenomic changes as well as DNA damage. Unlike hiPSC-derived neurons, directly converted neurons also generate both 3R and 4R forms of tau, which are necessary to form NFTs. However, participants noted that direct conversion protocols are difficult, and few labs have the expertise to successfully convert neurons directly from fibroblasts; directly converted neurons also cannot survive
shipment to other laboratories. Direct conversion also creates a limited resource; although fibroblast cultures can be expanded, they lose aging phenotypes over time. In addition, direct conversion protocols do not yet exist for astrocytes or MGLs. Participants suggested that direct conversion of neural cell types, especially MGLs, may generate cells that behave more similarly to their in vivo counterparts, enabling more accurate in vitro AD models.
Appendix 1: Workshop Agenda

Day 1: April 27, 2022

11:00 AM  Welcome and Opening Remarks  
Amanda DiBattista, PhD, Division of Neuroscience, National Institute on Aging

11:15 AM  Overview of Meeting

SESSION I:  Highlighting the Opportunities and Challenges in Modeling Basic Mechanisms of Brain Aging In vitro
SESSION Chair: Li-Huei Tsai, PhD, Massachusetts Institute of Technology

11:30 AM  Interrogating Alzheimer’s disease pathogenesis using iPSCs: delineation of aspects of biology captured and not captured  
Tracy Young-Pearse, PhD, Harvard Medical School

11:50 AM  Modeling multicellular interactions in AD brains using induced pluripotent stem cells  
Li-Huei Tsai, PhD, Massachusetts Institute of Technology

12:10 PM  3D brain-like tissue cultures from patient-derived iPSCs develop Alzheimer’s disease-related phenotypes  
Giuseppina Tesco, MD, PhD, Tufts University

12:30 PM  Break

12:50 PM  Roundtable Discussion with Speakers and Participants

1:20 PM  Development of IPSC resources to address Alzheimer’s and related dementias  
Mark Cookson, PhD, NIA Intramural Research Program

1:40 PM  Using stem cells to explore the genetics underlying brain disease  
Kristen Brennand, PhD, Yale School of Medicine

2:00 PM  Modeling Alzheimer’s disease as a genetically heterogenous innate immune disorder  
Rudolph Tanzi, PhD, Harvard Medical School

2:20 PM  Break

2:40 PM  Day One Wrap-Up Comments and Preview of Day Two
Day 2: April 28, 2022

11:00 AM   Welcome

**SESSION II:** Exploring the Challenges and Potential Value of Modeling Basic Mechanisms of Brain Aging and AD *In silico*
Session Chair: William Lytton, MD, SUNY Downstate Health Sciences University

11:10 AM   Multiscale modeling of dementias, from molecules to Stroop
*William Lytton, MD, SUNY Downstate Health Sciences University*

11:30 AM   Redundancy and realism in biological vs artificial neural networks
*Cian O’Donnell, PhD, Ulster University*

11:50 AM   Multiscale computational modelling in brain ageing and disease: from intracellular networks to cell-cell interactions in the tissue
*Antonio del Sol, PhD, University of Luxembourg*

12:10 PM   Break

12:30 PM   An in-silico lens into hippocampal learning and memory
*Yuri Dabaghian, PhD, University of Texas Health Science Center at Houston*

12:50 PM   FAIR resources and infrastructure for AD target discovery and validation
*Anna Greenwood, PhD, Sage Bionetworks*

1:10 PM   Roundtable Discussion with Speakers and Participants

**SESSION III:** Consideration of the Ethical Implications of Modeling Mechanisms of Brain Aging and AD *In vitro* and *In silico*
Session Chair: Matt Sutterer, PhD, Division of Neuroscience, National Institute on Aging

1:40 PM   Modeling basic mechanisms of brain aging: Re-invent the wheel of research ethics or add new spokes?
*Insoo Hyun, PhD, Harvard University*

2:00 PM   The ethical dilemma of brain models
*Henry T. Greely, JD, Stanford University*

2:20 PM   Roundtable Discussion with Speakers and Participants

2:50 PM   Break
SESSION IV: Final Panel Discussion and Wrap-Up  
**Session Chair:** Amanda DiBattista, PhD, Division of Neuroscience, National Institute on Aging

3:00 PM Discussion of Overall Research Gaps and Opportunities for Progress