Project Planning

InT BioHackathon | Team 24 | Mateo Umaguing, John Walkiewicz, Logan Umaguing

Project: Exploring differential effects of gamma stimulation on Alzheimer's Disease (AD) rodent models.

Research Proposal

2-8 page typed written work, which includes:

- Abstract
- Introduction (background, significance, current literature, knowledge gap)
- Methods
- Justification for the methods used
- Expected results
- Discussion

Additional graphs

Any code/scripts/packages used

Hardware

1-3 page typed written summary

- Abstract
- Introduction (background, significance, current literature, knowledge gap)
- Problem statement
- Methods: Solution design and code design
- Discussion

Idea [25]			Method / Design / Product [35]					Track Scores [35]						Presentation		Bonus	TOTAL	Average
9	9	5	9	4	4	10	4	4	5	15	5	5		5	4	5	102	
7	7	4	5	3	4	6	3	4	4	9	4	4		3	4	3	74	88.000

Research Proposal [35]	**Feasibility**	
	The proposed methodology is feasible to carry out within a few months given appropriate resource.	**5**
	Reproducibility	
	Reproducible and feasible within the scope of the proposed study.	**5**
	Justification	
	The proposed methodology is supported by literature.	**10**
	[ADD] The proposal demonstrates a thorough understanding of the field and relevant techniques.	**5**
	Expected results	
	Expected results are presented clearly with justification.	**5**
	Future Work	
	Future work is outlined with justification.	**5**

Software/Hardware [35]	**Completion**	
	A live/recorded demo of the Minimum Viable Product is available with no bugs.	**5**
	Most of the proposed functionality is achieved.	**10**
	Market value	
	The product has potential to be commercialized or used in the real world.	**5**
	Each of the following criteria is worth 5 marks. The highest three scores are added.	
	Functionality: The code performs its intended function with no impactful bugs.	**5**
	Code quality: The code is clear, concise and commented.	**5**
	Cutting edge: Algorithms implemented are complex, cutting edge (doesn't have to be original) and appropriate for the proposed solution.	**5**
	Originality: The code uses creative methods to implement a relatively new and complex function or algorithm.	**5**

Question

How does gamma stimulation help Alzheimer's Disease (AD) patients?

Our INITIAL "Known's"

- Alzheimer's patients show improvements in symptoms in response to gamma stimulation.
- Optogenetic stimulation of rodents can increase the gamma band power.
- Rodents with AD model show improvements in symptoms in response to gamma stimulation.
- Gamma activity is correlated with parvalbumin-positive (PV+) interneuron activity.
  - PV+ interneuron activity is GABAergic

My Questions

- Where are the gamma-correlated PV+ interneurons? Are they in the CA1?
- If these are IPSPs, is a region being suppressed?
- What does gamma stimulation exactly do at each system level? Is it really a panacea for all of these AD symptoms?
- Which regions have the most AB build-up? How is it caused?
- What is going on during gamma stimulation trans-regionally?
- Can targeted non-invasive brain stimulation (NIBS) be optimized to treat AD?
- What is the correlation between gamma activity, PV+ interneurons, GABA, and microglia?
- Does stimulating different regions lead to different cognitive changes in different behavioral assays/activities?
  - During decision-making
  - During fear conditioning
  - During learning/memory dependent tasks
  - During operant conditioning
  - During spatial awareness tasks
- What do PV+ interneurons do?
- Where do we go from here?

Model

1. Beta-amyloid proteins accumulate -> activation of microglia - microglia release inflammatory molecules and try to clear the AB plaques. Chronic activation of microglia is bad.
2. Tau proteins get abnormally modified and become neurofibrillary tangles -> disruption of normal functioning of neurons
3. Astrocytes regulate AB levels therefore their dysfunction can impair the clearance process

Symptoms and corresponding regions of the brain

1. Memory loss (hippocampus)
2. Confusion and disorientation (PFC/ACC, motor cortex)
3. Language and communication (PFC/Broca's/Wernicke's areas, auditory cortex)
4. Poor judgment & decision-making (PFC/ACC/OFC)
5. Challenges with problem-solving & planning (PFC/OFC)
6. Personality and mood changes (PFC)
7. Loss of initiative and motivation (PFC)
8. Agitation and aggression (amygdala)
9. Wandering and getting lost (hippocampus)

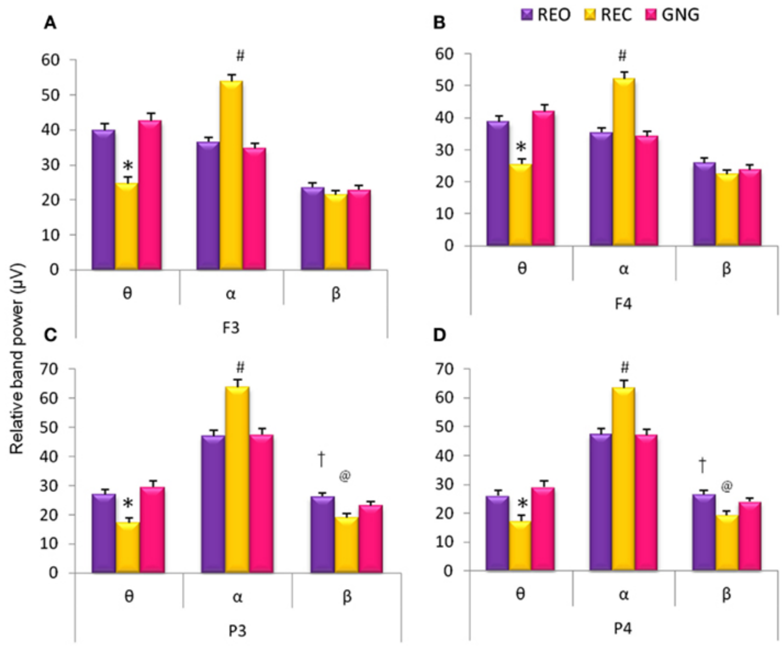
Answers to My Questions and Important Info

- 40 Hz opto + light flickering, inducing fast-spiking interneuron activity in visual cortex can reduce AB levels and mitigate plaque load in CA1 region of hippocampus by approximately 50% (Iaccarino et al. 2016)
    - transgenic 5XFAD mouse model used
    - TauP301S mouse model used and stim resulted in reduction of p-tau accumulation in visual cortext following daily exposure to 40 Hz flickering light stimulation for 1 h (Adaikkan et al. 2019)
- 20 Hz and 80 Hz stimulation did not have the same effects (Iaccarino et al. 2016)
- 1-h daily exposure to 40 Hz auditory stimulation for 1 week results in AB reduction in hippocampus and auditory cortex of 5XFAD mice (Martrorell et al. 2020)
    - leads to cognitive and behavioral improvement in hippocampus and auditory cortex
    - **chemogenetics** is possible if we target PV+ interneurons in hippocampus
- 1 h daily 40 Hz light flickering for 30 days increased circadian locomotor activity and promoted expression of proteins involved with AD pathological cascade (Yao et al. 2020)
    - increased gamma activity within visual cortex
    - reduced AB and p-tau production in hypothalamus observed
- 40 Hz light flickering and physical exercise on 3xTg murine model -> reduction of p-tau and AB in the hippocampus along with improvements in spatial learning, memory, and mitochondrial function. Best treatment was combination of exercise and 40 Hz light flickering (Park et al. 2020).
- PV+ interneurons

Tentative Experimental Design

Colors represent different stages of the experiment.

1. Verify that optogenetic stimulation can induce gamma oscillations. This can be done with an optetrode. This stimulation will be referred to as opto-gamma-stimulation (OGS)
2. Use histology to see what regions are affected (fMRI)
3. Test OGS on mice during the ___ behavioral/cognitive assay (choose from T-Maze, spatial memory, open field, tail-suspension, fear conditioning assays, operant conditioning assays and use DeepLabCut).
4. Test OGS for different durations in different areas (remember to include test vs. sham).
5. Verify that chemogenetic stimulation can induce gamma oscillations. This can be done with a viral vector and LFP recordings. This stimulation will be referred to as chemo-gamma-stimulation (CGS)
6. Test CGS on mice during the ___ behavioral/cognitive assay (choose from above).
7. Apply AD models to rodents.
8. Use histology, imaging, or other behavioral/cognitive assays to assess efficacy of applied AD models.
9. Test OGS in different time segments, durations, and areas for non-AD-OGS, non-AD-sham, AD-OGS, and AD-sham mice. This will be experimenter-blind, thus labels will be applied by the lab technician. The type of AD model applied can be another factor to observe.
10. Ascertain the most statistically significant treatments (time of stimulation, duration, region) with a Graeco-Latin square design.
11. Verify with histology that targets were correctly activated/inhibited.
12. Ca^{2+} individual neuron imaging analysis with UCLA Miniscopes
    a. proportions of regions active
    b. connectivity between regions
    c. histology to observe targeted cells
13. Test effects of LIFUP
    a. rodents: see if gamma band power can be increased with LFP recordings and or calcium imaging (fiber photometry,
    b. humans: see if gamma band power can be increased with EEG recordings



Predictions
The following behaviors may be measured with DeepLabCut and BehaviorDEPOT.

T-Maze AD:
- Impaired learning of 3v1 reward differential
- Impaired spatial memory
- LR preference
- Decreased speed
- Micropauses
- Vicarious trial-and-error

Open Field AD:
- Less exploratory behavior, more % time on the edges vs. center

Tail-Suspension AD:
- Less struggling

Foot-Shock AD:
- Impaired learning
- Reduced responsiveness to shock

Sleep AD:
- ___ rhythms
- ___ duration of sleep
- other assays?

Other things to look out for:
- Social behavior among colony
- ‘Forgetfulness’
- ‘Confusion’
- Lifestyle
- Grooming
- Alertness
- ‘Aloof-ness’
- Spatial un-awareness
- Impaired problem solving
- Bladder control
- Motor Control
- Mouth

Methods
- Optetrodes
- Histology
- UCLA Miniscopes

ChatGPT’s Explanation of Alzheimer’s
Prompt: Explain the relationship between Alzheimer's disease, glia, beta-amyloid proteins, and tau proteins

Answer: Alzheimer's disease is a neurodegenerative disorder that primarily affects the brain, leading to progressive cognitive decline, memory loss, and impaired functioning. The exact cause of Alzheimer's disease is not fully understood, but it is believed to involve complex interactions between multiple factors, including the accumulation of abnormal proteins, such as beta-amyloid and tau.

Glia are a group of non-neuronal cells in the brain that play various supportive roles, including maintaining the overall health and functionality of neurons. In Alzheimer's disease, glial cells, particularly microglia and astrocytes, are involved in the disease pathology.

Beta-amyloid proteins are fragments of a larger protein called amyloid precursor protein (APP). In healthy individuals, APP is broken down and cleared from the brain. However, in Alzheimer's disease, beta-amyloid proteins accumulate abnormally and form plaques, which are sticky clumps of protein that disrupt normal brain function. These plaques are primarily composed of beta-amyloid peptides, specifically a longer form called beta-amyloid 42 (Aβ42).

Tau proteins, on the other hand, are naturally occurring proteins that are involved in stabilizing the structure of neurons. In Alzheimer's disease, tau proteins undergo abnormal modifications, such as hyperphosphorylation. As a result, they become tangled and form twisted fibers known as neurofibrillary tangles inside neurons. These tangles disrupt the normal functioning of neurons and contribute to their degeneration.

The relationship between these elements in Alzheimer's disease is complex. The accumulation of beta-amyloid proteins leads to the activation of microglia, the immune cells of the brain. Microglia, in response to beta-amyloid, release inflammatory molecules and try to clear the beta-amyloid plaques. However, chronic activation of microglia and sustained inflammation can be detrimental to neuronal health and contribute to neurodegeneration.

Astrocytes, another type of glial cell, also play a role in Alzheimer's disease. They are involved in regulating the levels of beta-amyloid proteins and promoting their clearance from the brain. Dysfunction of astrocytes can impair this clearance process, leading to increased beta-amyloid accumulation.

Moreover, the accumulation of beta-amyloid plaques and neurofibrillary tangles composed of abnormal tau proteins can trigger a cascade of events that further damage neurons, disrupt neuronal communication, and contribute to the cognitive decline observed in Alzheimer's disease.

It's important to note that the exact mechanisms underlying Alzheimer's disease are still being actively researched, and there is ongoing debate and investigation into the precise roles of glia, beta-amyloid, and tau in the disease progression. However, these elements are known to be major players in the pathogenesis of Alzheimer's disease and are important targets for potential therapeutic interventions.

Prompt: What are the main cognitive and behavioral symptoms of Alzheimer's Disease?

Answer: Alzheimer's disease is characterized by a range of cognitive and behavioral symptoms that progressively worsen over time. The specific symptoms and their severity can vary among individuals, but here are some of the main cognitive and behavioral manifestations typically associated with Alzheimer's disease:

Memory loss: One of the earliest and most prominent signs of Alzheimer's is the impairment of short-term memory. Individuals may have difficulty remembering recently learned information, forget important dates or events, and repeatedly ask the same questions.

Confusion and disorientation: People with Alzheimer's may become disoriented in familiar surroundings, have trouble recognizing people, places, and objects, and may lose track of time.

Language and communication difficulties: Individuals may experience difficulty finding the right words, following conversations, or expressing their thoughts. They may also have trouble understanding written or spoken language.

Poor judgment and decision-making: Alzheimer's can impair a person's ability to make sound judgments and decisions. They may exhibit poor financial management, fall victim to scams or fraud, and struggle with problem-solving tasks.

Challenges with problem-solving and planning: The disease can make it difficult for individuals to perform complex tasks that require planning, organizing, and following through with a series of steps.

Personality and mood changes: Alzheimer's can lead to changes in personality, mood swings, and emotional instability. Individuals may become withdrawn, irritable, anxious, or exhibit uncharacteristic behaviors.

Loss of initiative and motivation: People with Alzheimer's may lose interest in activities they previously enjoyed, lack motivation, and have difficulty initiating and completing tasks.

Agitation and aggression: In some cases, individuals with Alzheimer's may exhibit restlessness, agitation, verbal or physical aggression, and disruptive behaviors.

Wandering and getting lost: As the disease progresses, individuals may wander aimlessly, often becoming disoriented and at risk of getting lost even in familiar environments.

It's important to note that the symptoms of Alzheimer's disease can vary from person to person, and the rate of progression can differ as well. If someone is experiencing cognitive or behavioral changes that concern them or their loved ones, it is advisable to consult with a healthcare professional for a thorough evaluation and diagnosis.

Prompt: Is there a particular region that has the most beta-amyloid protein build-up in Alzheimer's?

Answer: In Alzheimer's disease, beta-amyloid protein build-up typically occurs in specific regions of the brain. The most affected region associated with beta-amyloid deposition is the cerebral cortex, which plays a critical role in cognitive functions such as memory, language, perception, and thinking. Within the cerebral cortex, the beta-amyloid plaques tend to accumulate in certain areas, including the neocortex, hippocampus, and entorhinal cortex.

The neocortex is the outer layer of the cerebral cortex and is involved in higher-order brain functions. It is divided into different regions responsible for various cognitive processes. In Alzheimer's, the neocortex, particularly the association areas involved in memory and cognition, is often heavily impacted by beta-amyloid plaques.

The hippocampus, which is vital for the formation and retrieval of memories, is another region significantly affected by beta-amyloid in Alzheimer's disease. The accumulation of plaques in the hippocampus disrupts its normal functioning and contributes to memory impairment, one of the hallmark symptoms of the disease.

The entorhinal cortex, located in the medial temporal lobe, is crucial for memory formation and the connection between the hippocampus and other cortical regions. It is one of the earliest regions affected by beta-amyloid pathology in Alzheimer's disease.

It's important to note that while these regions are particularly susceptible to beta-amyloid accumulation, other brain regions can also be affected as the disease progresses. The spread of beta-amyloid pathology throughout the brain contributes to the widespread cognitive decline observed in later stages of Alzheimer's disease.

Prompt: What do PV+ interneurons have to do with gamma oscillations in the brain?

Answer: PV+ interneurons, also known as parvalbumin-positive interneurons, play a crucial role in generating gamma oscillations in the brain. Gamma oscillations are rhythmic patterns of neural activity that occur in the frequency range of approximately 30-100 Hz and are involved in various cognitive processes, including perception, attention, memory, and information integration.

PV+ interneurons are a subtype of inhibitory interneurons found in the neocortex and hippocampus, among other brain regions. They release the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and are known for their fast-spiking properties and precise control of neuronal firing.

The generation of gamma oscillations involves a balance between excitatory and inhibitory neural activity. PV+ interneurons play a critical role in this process by regulating the timing and synchronization of excitatory pyramidal neurons. Here's how PV+ interneurons contribute to gamma oscillations:

Synchronization: PV+ interneurons receive inputs from multiple pyramidal neurons and form extensive connections with them. They can synchronize the activity of pyramidal neurons by inhibiting them at specific time points. This synchronization helps generate coherent oscillatory activity in the gamma frequency range.

Feedforward inhibition: PV+ interneurons can exert feedforward inhibition on pyramidal neurons. When a group of pyramidal neurons become active, PV+ interneurons can rapidly inhibit them, preventing excessive excitation and maintaining the balance between excitation and inhibition. This inhibition leads to the rhythmic pattern characteristic of gamma oscillations.

Generation of network resonance: PV+ interneurons can create network resonance by generating rebound excitation. After inhibiting pyramidal neurons, PV+ interneurons experience a brief refractory period. When the inhibition is lifted, the rebound excitation of pyramidal neurons can occur, contributing to the synchronized firing necessary for gamma oscillations.

By their ability to provide inhibitory control and synchronization, PV+ interneurons help shape the timing and coherence of neural activity, enabling the generation of gamma oscillations.

These oscillations, in turn, facilitate the coordination of information processing across brain regions and are thought to be critical for cognitive functions.

Prompt: Where do PV+ interneurons send signals in the brain?

Answer: PV+ interneurons, also known as parvalbumin-positive interneurons, send signals to various targets within the brain. These interneurons are known for their widespread connectivity and play a crucial role in regulating neural circuit activity. Here are some of the main targets to which PV+ interneurons send signals:

Local pyramidal neurons: PV+ interneurons form synapses with nearby pyramidal neurons within the same brain region. These connections allow for local inhibition and the control of excitatory output from pyramidal neurons.

Other interneurons: PV+ interneurons can also target and inhibit other interneuron populations. This inhibition helps to regulate the balance of excitation and inhibition within neural circuits.

Dendrites of pyramidal neurons: PV+ interneurons can synapse onto the dendrites of pyramidal neurons. By targeting dendritic compartments, PV+ interneurons play a role in regulating the integration of synaptic inputs and the overall computational properties of pyramidal neurons.

Axon initial segments: PV+ interneurons can provide direct inhibition to the axon initial segments of pyramidal neurons. This inhibition can control the initiation and propagation of action potentials in pyramidal neurons.

Thalamus: PV+ interneurons have been found to project to the thalamus, a crucial relay station that plays a role in sensory processing and information transmission between different brain regions. This projection from PV+ interneurons to the thalamus can modulate thalamic activity and contribute to the regulation of sensory processing.

It's important to note that the specific targets of PV+ interneurons can vary depending on the brain region and the specific circuit in question. PV+ interneurons exhibit diverse connectivity patterns, allowing them to exert precise inhibitory control and shape the activity of neural networks. Their broad connections enable them to modulate the information flow and excitability within brain circuits, playing a critical role in neural computation and network dynamics.

Prompt: Is there any relationship between PV+ interneurons and beta-amyloid protein build-up?

Answer: Research suggests that there may be a relationship between PV+ interneurons and beta-amyloid protein build-up in Alzheimer's disease, although the exact nature of this relationship is still being investigated. Here are some findings and hypotheses regarding the potential link between PV+ interneurons and beta-amyloid:

PV+ interneuron dysfunction: Studies have shown that in Alzheimer's disease, there is a selective vulnerability of PV+ interneurons in brain regions affected by beta-amyloid pathology. PV+ interneurons exhibit reduced numbers, altered morphology, and impaired function in the presence of beta-amyloid plaques.

Disrupted inhibitory control: PV+ interneurons play a crucial role in providing inhibitory control within neural circuits. Dysfunction or loss of PV+ interneurons may lead to an imbalance of excitation and inhibition, resulting in network hyperexcitability. This imbalance could contribute to the cognitive and neuronal dysfunction observed in Alzheimer's disease.

Impact on network activity: PV+ interneurons regulate the synchronization and timing of neural activity, including the generation of gamma oscillations. Disrupted PV+ interneuron function due to beta-amyloid accumulation may impair gamma oscillations, which are critical for information processing and cognitive functions. Altered network activity may contribute to cognitive deficits observed in Alzheimer's disease.

Beta-amyloid effects on PV+ interneurons: Beta-amyloid itself can have direct effects on PV+ interneurons. In animal models and in vitro studies, beta-amyloid exposure has been found to lead to increased oxidative stress, calcium dysregulation, and altered synaptic function in PV+ interneurons. These effects could contribute to their dysfunction and loss in Alzheimer's disease.

Interactions with tau pathology: In Alzheimer's disease, both beta-amyloid and tau proteins are involved in the pathology. Some studies suggest that tau pathology may exacerbate the impact of beta-amyloid on PV+ interneurons. Tau protein accumulation in PV+ interneurons could lead to additional cellular dysfunction and contribute to the disruption of inhibitory control.

While the relationship between PV+ interneurons and beta-amyloid in Alzheimer's disease is still being elucidated, these findings suggest that the dysfunction and loss of PV+ interneurons may contribute to the pathological processes and cognitive impairments associated with beta-amyloid accumulation. Further research is needed to fully understand the underlying mechanisms and potential therapeutic implications of this relationship.

Certainly! Here's an outline for an introduction section for your proposed study on exploring the differential effects of gamma stimulation on Alzheimer's Disease (AD) rodent models:

I. Introduction

A. Background Information
1. Briefly introduce Alzheimer's Disease (AD) as a progressive neurodegenerative disorder affecting a significant proportion of the aging population.
2. Highlight the cognitive impairments, memory loss, and behavioral disturbances associated with AD.

3. Discuss the current understanding of AD pathophysiology, including the accumulation of beta-amyloid plaques and neurofibrillary tangles, as well as synaptic dysfunction and neuronal loss.

B. Therapeutic Approaches for AD
1. Describe the current state of AD treatment and management, emphasizing the need for effective interventions.
2. Discuss the limitations and challenges associated with existing pharmacological interventions, such as modest efficacy and potential side effects.
3. Introduce the concept of non-pharmacological interventions as alternative or adjunctive therapies for AD, focusing on their potential to enhance neuronal function and cognitive abilities.

C. Role of Neural Oscillations in AD
1. Explain the significance of neural oscillations (brainwave patterns) in normal brain function and cognition.
2. Discuss alterations in neural oscillations observed in AD, particularly the disruption of gamma oscillations (30-100 Hz) associated with cognitive deficits.
3. Highlight evidence linking gamma oscillation abnormalities to the pathophysiology of AD, including impaired synaptic plasticity and neuronal communication.

D. Gamma Stimulation as a Potential Therapeutic Strategy
1. Introduce gamma stimulation (transcranial alternating current stimulation or other relevant techniques) as a non-invasive brain stimulation method.
2. Explain how gamma stimulation can modulate neural oscillations and potentially restore gamma synchronization in AD.
3. Summarize previous preclinical and clinical studies exploring the effects of gamma stimulation on AD-related outcomes, highlighting promising findings but acknowledging the need for further investigation.

E. Study Objective and Hypothesis
1. Clearly state the objective of the proposed study: to investigate the differential effects of gamma stimulation on AD rodent models.
2. Formulate a hypothesis based on the existing literature and the specific research question(s) of the study.

F. Study Outline
1. Provide a brief overview of the structure of the remaining sections of the paper.
2. Outline the methods employed to address the research question(s), including the choice of AD rodent models, gamma stimulation parameters, experimental design, and outcome measures.
3. Mention the significance of the study in advancing our understanding of gamma stimulation as a potential therapeutic approach for AD and its implications for future research and clinical applications.

By following this outline, you can effectively introduce the background, rationale, and objectives of your study on exploring the effects of gamma stimulation on Alzheimer's Disease rodent models. Remember to adapt the outline to fit the specific details and context of your research.

People to contact:

- Istvan Mody
- Jun Chen
- Jessica Rexach
    - Immunology -> treating dementias
    - How glia and immune factors influence disease pathology in dementia
    - Microglia = immune cell in brain
    - “We discovered that there is a strong component of immune suppression beginning at very early disease stages in these dementias. We also discovered that dementia genes land in the biological pathways that are classically part of viral defense.”
- Chao Peng
    - Molecular and cellular mechanisms of neurodegenerative diseases
    - Pathological protein transmission
    - Neuron-glia interaction
- Jason Hinman
    - Molecular pathways -> therapeutics
    - ID’ing vascular dementia-prone patients
- Varghese John
    - New Chemical Entities = therapeutics for AD
    - DDL110 is an NCE that increases sAPPa to AB ratio
- Karen Gylys
    - Synaptic pathology in Alzheimer’s disease
    - CSF and Blood Biomarkers in Alzheimer’s disease
- Timothy Chang
    - Genetic and computational approaches
- Keith Vossel
    - Mechanisms and therapies that target tau protein
- Scott Wilke
- Laura DeNardo
- Gina Poe
- Martin Monti
- Nanthia Suthana
- Alexander Korb

ANYONE WHO WORKS IN

- Cognition
- Behavioral Health