FLICKER: Stimulating neural activity to improve blood flow and reduce amyloid: Path to clinical trials

NCT number: NCT03543878

Protocol date: November 4, 2019

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Investigators:

James J. Lah, MD, PhD
Department of Neurology
Emory University School
of Medicine

Co-Investigators:

Annabelle Singer, PhD
Assistant Professor
Coulter Dept. of
Biomedical Engineering
Georgia Tech & Emory
University

Allan I. Levey, MD, PhD
Department of Neurology
Emory University School
of Medicine

Sponsors:

Emory University
With funding provided by
Cognito Therapeutics

1. BACKGROUND AND SIGNIFICANCE

Alzheimer's Disease (AD)is a looming epidemic, with an urgent need for new therapies to delay or prevent symptom onset and progression. There is growing awareness that clinical trials must target stage-appropriate pathophysiological mechanisms to effectively develop disease-modifying treatments.[1] Advances in AD biomarker research have demonstrated changes in amyloid, brain metabolism, and other pathophysiologies prior to the onset of memory loss, with some markers possibly changing one or two decades earlier [2,3]. The brain region responsible for spatial navigation and memories of experience, the hippocampus, is one of the areas first affected in Alzheimer's disease (AD) and other memory disorders.[4,5]Researchby A. Singer has shown how coordinated electrical activity across many neurons in the hippocampus represents memories of experiences[6,7]and this coordinated activity fails in animal models of AD. The work also showed that stimulating neurons to produce a specific component of this activity, called gamma oscillations, reduces AD pathology. The goal of this proposal is to translate our discovery that stimulating specific patterns of neural activity is neuroprotective from rodents to humans using a non-invasive approach. This first study will establish the feasibility and tolerability of using this non-invasive method to drive gamma in humans. This research includes preclinical testing that will be used to design and justify a multi-site clinical trial to test this approach as a treatment for AD, for which there are currently no effective therapies.

Preclinical studies by A. Singer and collaborators recently reported a powerful new discovery in *Nature*: driving specific patterns of neural activity recruits the brain's immune system and reduces AD pathology.[8]In AD mice, deficits were found in 20-50Hz electrical neural activity that has long been linked to memory, attention, and perception, called gamma oscillations. Deficits in gamma oscillations occurred in presymptomatic mice, suggesting that they may contribute to early disease progression. Surprisingly, stimulating neurons to produce gamma oscillations ("drive gamma") mobilized the immune system to remove amyloid beta, a protein whose aggregation is thought to initiate neurotoxic events. Specifically, driving gamma recruited the primary immune cells of the brain, microglia, to increase engulfment of beta amyloid, resulting in 40% reduction in beta amyloid (Fig. 1).

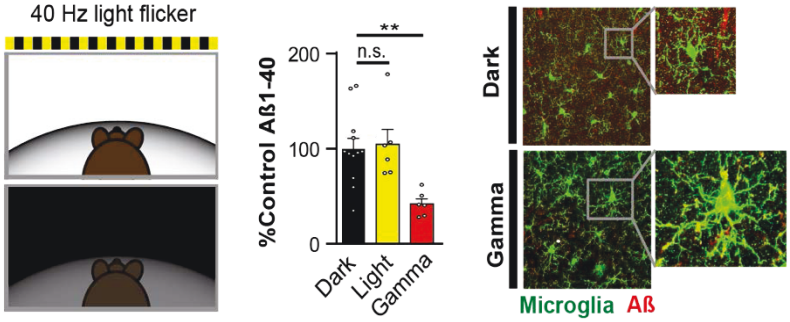

Figure 1: Stimulating brain circuits to produce gamma oscillations (left) reduces amyloid beta levels (center) and recruits microglia to take up amyloid (right).

In the animal studies, invasive optogenetics (which requires virus infection and fiber implants to drive neural activity with light) were used to drive gamma.[9,10]

To translate this discovery to humans, A. Singer and colleagues developed a method to drive gamma non-invasively: flickering lights at gamma frequency, like a strobe light but faster, to drive gamma oscillations in visual brain areas (Fig. 1). This simple non-invasive manipulation can be applied to humans to determine if this approach can be used therapeutically.

Blood flow through the brain is crucial for neural function and clearance of pathogens.[11-13]Blood flow decreases very early in Alzheimer's disease and this decrease is thought to lead to amyloid build up and functional deficits.[14,15]In preliminary studies, driving gamma leads to dilation of blood vessels even after manipulation of activity ceases. One hour after gamma light flicker ends, the diameter of small blood vessels is increased by 33% and pathogenic proteins appear co-localized with these vessels (Fig. 2). We hypothesize that this dilation of blood vessels contributes to the decrease in pathogenic proteins following gamma light flicker by increasing clearance via the blood steam. Accordingly, we will assess the effects of driving gamma on blood flow to reveal an entirely new effect of stimulating gamma oscillations. Stimulating activity to increase blood flow may reduce pathogenic proteins and could have broad implications for brain health.[13]

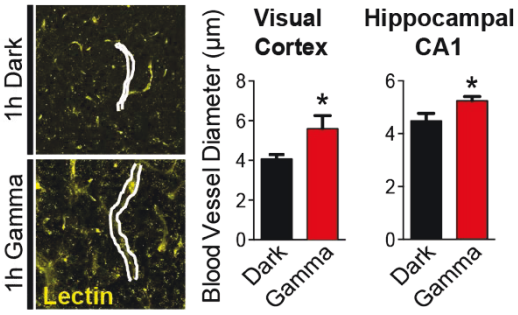

Figure 2: Gamma light flicker for 1 hour increases blood vessel diameter in visual cortex and hippocampus relative to dark controls.

Clinical and preclinical studies have demonstrated that gamma activity can be increased via exposure to several different types of sensory stimuli. Recent preclinical findings published in *Nature* have demonstrated that this increase in gamma activity both reduced the production of beta-amyloid plaques and increased the rate of clearance in the brains of mice[7]. Preliminary (unpublished) data using combined audio and visual sensory stimulation has demonstrated improvement in cognitive behavioral tasks in preclinical mouse models of AD following this intervention. Non-invasive sensory stimulation paradigms thus hold therapeutic promise for those individuals with mild cognitive impairment, Alzheimer's disease or related disorders.

The specific modalities have been chosen because they have been widely used in previous clinical research for a variety of goals, including the characterization and understanding of gamma brain activity in younger and older adults[16,17]. For example, the most efficient forms of visual stimulation for generating gamma activity (including properties such as frequency and intensity) have been identified[18,19]. For auditory stimulation, the test-retest reliability of its effect on gamma brainwave activity is high[6] and the gamma response induced by auditory "click-train" stimuli at 30-50 Hz has been proposed as a quantitative measure of hearing loss in old age, an indicator for neurodevelopment conditions such as schizophrenia[20-22]and neurodegenerative conditions such as Alzheimer's disease[23].

The urgent and essential next step of this work is to translate these discoveries to humans in a clinical investigation. This pilot study in humans will focus on feasibility and safety. Our central hypothesis is that driving gamma via sensory flicker will be feasible and tolerable. Our secondary hypothesis is driving gamma alters the brain's immune cells and amyloid beta processing. The primary outcomes will be feasibility and tolerability. Exploratory measures will include amyloid and immune signals in the CSF and brain and alterations in cerebral blood flow, as seen through arterial spin-labeling (ASL) magnetic resonance imaging (MRI).

2. SPECIFIC AIMS

2.1*Aim 1. Assess the safety, and tolerability of gamma flicker and subject compliance.* We will determine in 10 subjects with a diagnosis of MCI whether flicker exposure for 1 hour every day is well tolerated and whether subjects comply with the study procedures. In addition, we will closely monitor the safety of this procedure and document the occurrence of any adverse events.

2.2*Aim 2. Assess effects of driving gamma on blood flow in patients with MCI.* Blood flow decreases very early in Alzheimer's disease and this decrease is thought to lead to amyloid build up and functional deficits.[14,15] We will determine if gamma flicker increases blood flow in Alzheimer's patients. We will perform these tests in approximately 10 patients with mild cognitive impairment (MCI) in collaboration with Dr.'s Lah and Levey and the Emory BHC/ADRC. These pilot studies will support our application for an NIH grant to test gamma flicker in Alzheimer's patients in a clinical trial.

2.3 Aim 3. Assess effects of driving gamma on amyloid beta levels and inflammation markers in patients with MCI. We will assess amyloid beta levels in the brain (florbetapir PET), cerebral spinal fluid (Aß42 Luminex assay), the current gold standards in the field, and in the retina (Neurovision), a new approach currently being validated at the Emory Brain Health Center. We will also assess inflammatory markers in the cerebral spinal fluid (OLink inflammatory panel). We will perform these tests in 10 patients with mild cognitive impairment (MCI), as determined by clinical or research assessment using local standards at the Emory Brain Health Center (BHC)/ Alzheimer's Disease Research Center (ADRC).

3. PRELIMINARY DATA

3.1 *Current work in humans*– Cognito Therapeutics has licensed the technology from MIT to transition this work to humans. The company will provide the flicker devices for

conducting the study here at Emory. The device is similar to sunglasses and is both comfortable and easy to use (Figure 3, *redacted*).

Initial human studies of patients with cognitive impairments have demonstrated that sensory stimulation can be safely delivered and is well-tolerated. Three clinical studies have been sponsored by Cognito Therapeutics, which have been conducted under IRB approval (non-significant risk designation) and with good clinical practice (GCP) using a combination of non-invasive sensory stimulation systems in patients with cognitive impairments. These studies used three sensory stimulation modalities: visual, auditory, and tactile. For the visual and auditory stimulation, the Visual Flicker Unit (COG-VSU001) and Auditory Click Unit (COG-ASU001) were utilized, which have similar output light intensity, sound volume, repetition rate (40 cycles/second), and duty cycle to the Gamma Sense Stimulation System that will be used for this study. For the tactile stimulation, transcutaneous electrical nerve stimulation (TENS) was delivered to the palm of the hand using the Peripheral Nerve Stimulation Unit (COG-NSU001). Tactile stimulation is not included in the clinical investigation described by this protocol.

redacted

4. EXPERIMENTAL DESIGN AND METHODS

4.1 Overview: For this 9-week study, 10 subjects with mild cognitive impairment (amnestic or multi-domain subtypes) will be randomly assigned to two study arms. Although all participants will receive Flicker exposure, half of the participants will receive the exposure (driving gamma) during the entire study period (8 weeks of Flicker) while the other half of the participants will receive Flicker exposure for half of the study period (2nd 4-weeks).During the 9-week course of the study, participants will undergo venous blood draws and lumbar puncture for biomarker analyses at baseline, midpoint and at 9 weeks. At the study week 4 time point, the participants currently undergoing Flicker exposure will continue with their sessions while at week 4, those initially randomized to non-exposure will initiate Flicker exposure. The primary goals are to measure safety and FLICKER tolerance while verifying the intended response. Secondary objectives include monitoring possible effects of Flicker exposure on clinical findings and biomarkers of AD progression, including CSF amyloid, tau, and phospho-tau, and volumetric MRI, as well as PET (florbetapir) and arterial spin labeling MRI and retinal amyloid changes.

4.2 Sample Size and Power. This is a pilot study designed to assess feasibility and tolerability in the patient population. Although the sample size of 10 subjects will allow us to make certain claims about tolerability, we do not anticipate generating clinically meaningful,

broadly applicable results from this data. Thus, our sample size is driven by the need to collect pilot data that will position us to develop a clinical trial, if warranted, based on the technology described here.

4.3 Flicker tolerance to date. For studies conducted to date by Cognito Therapeutics, a total of 17 participants have undergone daily periods of sensory stimulation for up to one hour (the study paradigm planned for this work). These subjects include young adults, older adults, and older adults with cognitive impairments. These studies have helped establish the feasibility of sensory stimulation with regards to safety, comfort, and tolerability of the exposure in patients with mild-to-moderate cognitive impairments. No device or exposure-related adverse events have been reported during this study.

4.4 Future research samples. Per participant consent, blood, cell lines and cerebrospinal fluid samples will be stored indefinitely and may be used for future investigation, potential development of new biomarkers, and potential treatments for Alzheimer's disease. Samples may also be used to deal with future safety issues for Mild Cognitive Impairment or Alzheimer's disease that are not presently known. These stored samples may also be used for genetic (DNA) research.

5. RESEARCH STUDY PERSONNEL

5.1 Principal Investigator (PI)for the study is James J. Lah, MD, PhD., Associate Professor, Department of Neurology. The PI will delegate authority for all study procedures performed as noted on the Delegation of Authority log.

5.2 Co-Investigators Annabelle Singer, Ph.D. Assistant Professor Coulter Dept. of Biomedical Engineering Georgia Tech & Emory University and Allan Levey, MD, PhD

5.3 Research Coordinator Experienced research coordinator(s) will be utilized to coordinate the research study.

5.4 Psychometrician Psychometric tools will be administered by research staff certified to conduct the specific tests utilized and who are blinded to adverse events/outcomes.

5.5 Blinded clinical rater Clinical rating tools will be administered by research staff who are certified to conduct the instruments and who are blinded to adverse events/outcomes.

5.6 Statistician. A statistician with expertise in data analysis for clinical studies of this type will be engaged to help with data analysis.

6. SAFETY MONITORING AND ADVERSE EVENT REPORTING

6.1 Internal Safety Monitoring Subjects will be monitored remotely to determine whether they are performing the proper Flicker exposure at home. The study coordinator will call 1 time during week 1 of the exposure phase to check on the participants. During the first week, the study coordinator will also visit the participant at home to observe the session. During weeks 2 – 4 of the study, the study coordinator will check-in with participants weekly by telephone. Subjects in the Non-exposure/Flicker group will be monitored in the same manner throughout their 4 weeks of Flicker exposure.

Internal monitoring will be performed by the Investigators who will review data from the study visits and review all aspects of patient safety. Serious and unexpected adverse events and/or unanticipated problems involving risks to participants or others will be reviewed and reported to the Emory IRB, as per the Emory IRB policies and procedures, by the research coordinator in collaboration with the investigators.

6.2 Emory University Investigational Review Board (IRB) policies and procedures will be employed:

Unanticipated Problems involving Risks to Participants or Others (UPs): The PI will report to the IRB (using any IRB specified forms, as applicable) any UP in accordance with the timetable set forth in the provision below entitled Timetable for Reporting. The PI will advise the Emory IRB of: (a) the relationship between the problem and the intervention or study protocol; (b) whether or not a protocol change is necessary to reduce risk; and (c) whether the information about the problem affects the informed consent process.

Serious Adverse Events: At the time of renewal for the study, the PI will report to the Emory IRB a summary of the serious adverse events related to the research that occurred in the previous approval period. If adverse events occur at a greater frequency, severity, or duration than was previously anticipated, those become unanticipated and are promptly reportable.

Protocol Deviations: The PI will report to the Emory IRB any circumstances of which the PI becomes aware per which there has been a substantive deviation from the protocol that adversely affected at least one of the following: 1) the rights, welfare, or safety of subjects; 2) the subjects' willingness to continue participation; or 3) the integrity of the research data. Deviations that are reported as protocol modifications undertaken to eliminate apparent immediate hazards to Human Subjects do not need to be reported twice. The IRB will evaluate reported protocol deviations to determine any action to be taken or other reporting requirements that need to be fulfilled.

Protocol Modifications: The PI will request approval of any changes (including, but not limited to, any changes in research personnel) that the PI plans to make to a Research protocol, which has already been approved by the Emory IRB. Except in cases in which the change must be made to eliminate apparent immediate hazards to Human Subjects**,** the Emory IRB must approve of the modification **before** it can be initiated.

Non-Compliance: The PI shall notify the IRB of any circumstances of which the PI becomes aware of failure to follow Emory IRB Policies &Procedures, federal regulations, or other applicable laws. This report shall be made to the IRB as soon as possible after the non-compliance occurs. The IRB will work closely with the PI to develop a reasonable corrective and preventive action plan that can be implemented by the study team.

7. PATIENT SELECTION

7.1 Recruitment Plan. The Emory ADRC has a large clinical practice and clinical research registry of subjects interested in participating in research studies. As of December 2017, the ADRC registry has an enrollment of 447 participants, among them 110 with MCI. The MCI participants include 100 individuals with amnestic MCI (single or multiple domain). These ADRC participants have annual research evaluations that include detailed cognitive assessments, and all have consented to be contacted for opportunities to participate in research studies. In addition to the existing pool of ADRC participants, the Emory memory disorders clinics, staffed by Drs. Levey, Lah, and other specialty-trained health professionals provide evaluation and treatment ~2000 patients annually, of whom ~500 are new visits (and ~100 MCI). Potential study subjects will be identified from those subjects being followed by the Emory Alzheimer's Disease Research Center and by the patients of the PIs. Other potential study subjects may be recruited if they contact the Emory ADRC specifically to find out about potential research studies that might be appropriate for either themselves or a family member.

7.2 Inclusion Criteria

1. Subjects must have a subjective memory concern as reported by subject, study partner or clinician.
2. Meets local criteria for diagnosis of MCI. Subjects with amnestic (single or multi-domain) will be eligible, as both subtypes of aMCI are at high risk for progression to AD.
3. Montreal Cognitive Assessment (MoCA)score $\geq$15. Exceptions may be made for subjects with less than 8 years of education at the discretion of the PI.
4. Clinical Dementia Rating = 0.5. Memory Box score must be at least 0.5.
5. General cognition and functional performance sufficiently preserved such that a diagnosis of AD cannot be made by the physician at the time of the screening visit.
6. Stable on medications for 4 weeks prior to initiation of study sessions.
7. Geriatric Depression Scale (GDS) ≤ than 6.
8. Male or female outpatients aged 50-90 (inclusive).
9. Able to hear without the use of hearing aids.
10. Study partner who lives with the participant and can provide a reliable assessment of the participant’s level of function, is available for all clinic visits, and can serve as a supervisor/monitor for the home-based Flicker sessions for the duration of the study.
11. Visual and auditory acuity adequate for neuropsychological testing.
12. Good general health with no diseases expected to interfere with the study.
13. Completed six grades of education or has a good work history (sufficient to exclude mental retardation).

14. Able to communicate in English with study personnel.
15. Able to understand the nature of the study and provision of written informed consent prior to conduct of any study procedures.
16. Willing to undergo repeated MRIs and PET scans. No medical contraindications to MRI.
17. Agrees to blood collection for APOE and biomarker testing.
18. Agrees to lumbar puncture over the course of the study for the collection of CSF.CSF levels of Ab42, total Tau, and Tau phosphorylated at threonine 181 consistent with underlying AD pathology according to established threshold values at Emory.

7.3 Exclusion criteria

1. Any significant neurologic disease other than MCI and suspected incipient AD, such as Parkinson's disease, multi-infarct dementia, Huntington's disease, normal pressure hydrocephalus, brain tumor, progressive supranuclear palsy, poorly controlled seizure disorder, subdural hematoma, multiple sclerosis, or history of significant head trauma followed by persistent neurologic deficits or known structural brain abnormalities.
2. Screening/baseline MRI scan with evidence of infection, large vessel infarction or other focal structural lesions that could account for the memory deficits. Subjects with multiple lacunes or lacunes in a critical memory structure are excluded.
3. Contraindication to MRI due to pacemakers, aneurysm clips, artificial heart valves, ear implants, metal fragments or foreign objects in the eyes, skin or body, or excessive weight.
4. Presence of clinically significant suicide risk based on the Investigator's judgment informed by a structured clinician interview. Any suicide attempt within the past 1 year of the screening visit is exclusionary.
5. Major depression, bipolar disorder as described in DSM-IV within the past 1 year, or history of schizophrenia (DSM-IV). Psychotic features, agitation or behavioral problems within the last 3 months which could lead to difficulty complying with the protocol.
6. History of alcohol or substance abuse or dependence within the past 2 years (DSM-IV criteria).
7. Known history of epilepsy or migraines, which may be exacerbated by study intervention.
8. History of narrow angle (acute angle) glaucoma.
9. Current use of warfarin or other blood thinners (exclusionary for lumbar puncture).
10. Inability to obtain initial CSF sample.
11. Current use of Namenda (memantine).
12. Current use of medications that lower seizure threshold, including Wellbutrin, Ciprofloxacin, Levofloxacin, Seroquel, Phenergan, and Sumatriptan.
13. Current use of anti-psychotic medication.
14. CSF profile inconsistent with underlying Alzheimer's Disease pathology.
15. Reasonable likelihood for non-compliance with the protocol or any other reason, in the opinion of the investigator, prohibits enrollment of subject into the study.

16. Exceptions to these guidelines may be considered on a case-by-case basis at the discretion of the protocol director.

7.4 Patient Numbering

Patients who have signed the informed consent form to begin screening procedures will be assigned a Subject Identification Number at that time. The participant will keep the same number throughout the study (a separate randomization number will not be provided).

7.5 Randomization

To achieve balanced patient groups, subjects will be randomly assigned on a 1:1 basis in a blinded fashion to the 8-week Flicker exposure or the 4-week Flicker exposure, stratified based on gender, and age.

8. STUDY ASSESSMENTS AND PROCEDURES

The study design and assessment schedule are presented in Table 1.

8.1 Informed Consent. The study will be discussed with the study subject and appropriate others (family, study partner) and if they are interested in learning more about the study they will be provided with a copy of the informed consent form to review in detail. The potential participant will be contacted at a later date to determine if they would like to be screened for possible study inclusion. If they are interested, a screening visit will be scheduled. Potential subjects are encouraged to discuss participation in this study with their family prior to screening for the study. In addition, they are encouraged to contact the office with any questions or concerns.

At the time of the screening visit, the consent form will be reviewed with the study subject and appropriate others, and all questions will be answered. Ample opportunity will be given for questions. If they are still interested in participating once all questions are answered to participant and study partner satisfaction, the consent is signed.

This study includes subjects who must score between >15 on the MoCA (Montreal Cognitive Assessment) and will therefore be experiencing mild cognitive impairment as a result of suspected prodromal Alzheimer's disease. Individuals with mild impairment will likely be able to provide informed consent. Capacity for consent will be determined by the study subject's ability to engage in a discussion regarding the study and their ability to understand what will be involved in study participation. The individual obtaining consent will review and discuss the research project and the consent document with the potential study participant and their family/study partner/legally authorized representative and will decide if they are able to understand the nature of the research, appreciate the experimental nature of the study, and understand the potential risks as well as alternatives to study participation.

In the event that the subject is not competent to provide consent, assent is obtained. A study subject's legally authorized representative will be present during the initial consenting process. Signed consent will be obtained from all participants and their study partners. The same process described previously will be employed in obtaining legally authorized

representative's consent, when deemed necessary. Consent will be obtained prior to conducting any screening assessments or procedures.

Participants under consideration for participation will have a diagnosis of MCI through the cognitive clinic or through consensus diagnosis as a participant in another of our research studies. Those who do not meet criteria for amnestic MCI will not be considered for participation.

8.2 Screening Visits.

Screening Visit 1a:

- Subjects will sign a written informed consent prior to any study-related procedures.
- Medical history, including review of medications
- Physical and neurologic exams
- Vital signs, including: blood pressure, heart rate, respiratory rate, temperature
- Height and weight
- Sensory function (see/hear)
- Cognitive Testing – Montreal Cognitive Assessment (MoCA)
- Flicker Tolerance Testing
- EEG
    - Flicker entrainment and exposure titration
- MR Imaging
    - If imaging is available within 6 months of screening visit 1a (including an arterial spin labeling [ASL] sequence), the screening MRI may not be performed

Screening visit 1a may take place on more than 1 day (e.g., MRI scan occurs on separate day). Subjects cleared for continued participation will undergo the following procedures at the second screening (**1b**). The tasks below may be performed on the same day, or may be performed on separate days. Screening 1b will occur within one calendar month of screening 1a.

Screening Visit 1b:

- Blood work
    - Inflammatory biomarkers
    - Metabolomics
    - Primary Panel for Inflammation, Oxidative, and Nitrosative Stress
    - APOE status (if unknown)
- Retinal Imaging
- If the subject has had a clinic or research LP within 6 months prior to visit 1b and CSF has been banked, no screening LP will be performed. The banked CSF will be used for analysis. In all other cases, an LP will be performed by an MD or NP following an overnight (no less than 6-hr) fast. CSF will be assessed for the following:

    - Aβ42, tau, phospho tau
    - Inflammatory Biomarkers
- Contact subject on the day after LP to inquire about any adverse events.

8.3 Baseline Visits. The baseline visit will occur within 1 calendar month of Screening visit **1b**. Patients that continue to meet eligibility requirements will undergo the following at visit **2a.**

Baseline Visit 2a:

- PET scan (florbetapir)

Baseline Visit 2b:

- Interim history review
- Medication review
- Adverse event review
- Confirm continued eligibility
- Vital signs, height & weight
- Physical and neurologic exams
- Cognitive testing/Questionnaires –
    - Category Fluency (Animals)
    - Benson
    - Trails A & B
    - Boston Naming Test (30 item)
    - Clinical Dementia Rating (CDR)
    - Neuropsychiatric Inventory (NPI)
    - Activities of Daily Living (FAQ)

- EEG
    - Flicker entrainment with exposure titration
    - EEG evaluation and induced activity
- Eye tracking task
- Randomization
    - Assigned to Flicker for 1st 4-weeks or 2nd 4-weeks
- Instructions for at-home Flicker sessions
    - Daily journal

8.4 Study Visits. Study visits at Emory may take place on more than 1 day to accommodate all the scheduled procedures (e.g., Visit 1 may be 2 days)

Week 1 (Flicker administration [will start in study week 4 for half of participants and procedures will be identical to those followed for the Flicker/Flicker group])

- Days 1 -7
    - In-home therapy
    - Telephone call/email contact
        - Assessment of adverse events

        - Answer any questions
    - Remote adherence monitoring
        - If session has not been completed by 2:00 pm, reminder call is placed
- Day 3, 4 or 5
    - In-home Observation visit

Weeks 2 – 4(Days 15 - 28)
- In-home therapy
- Telephone call/email contact
    - Assessment of adverse events
    - Answer any questions
- Remote adherence monitoring
    - If session has not been completed by 2:00 pm, reminder call is placed
- Flicker administration will continue until all assessments have been completed.

Midpoint Visit a:
- Interim history review
- Medication review
- Adverse event review
- Confirm continued eligibility
- Vital signs, height & weight
- Physical and neurologic exams
- Retinal Imaging
- PET scan (florbetapir)
- Cognitive testing/Questionnaires –
    - Clinical Dementia Rating (CDR)
    - Neuropsychiatric Inventory (NPI)
    - Activities of Daily Living (FAQ)
- EEG –
    - Flicker entrainment with exposure titration
    - EEG evaluation and induced activity
- Eye tracking task
- Instructions for at-home Flicker sessions (for those initiating Flicker)
    - Daily journal

Midpoint Visit b:
- LP
- MRI

End-of-study Visit a:
- Interim history review
- Medication review
- Adverse event review
- Confirm continued eligibility

- Vital signs, height & weight
- Physical and neurologic exams
- Cognitive testing/Questionnaires –
    - Clinical Dementia Rating (CDR)
    - Neuropsychiatric Inventory (NPI)
    - Activities of Daily Living (FAQ)
- PET scan (florbetapir)
- MRI
- EEG
    - Flicker entrainment with exposure titration
    - EEG evaluation and induced activity
- Eye tracking task
- Turn in journal/equipment if Flicker completed during second 4-week period

End of Study Visit b:

- Retinal Imaging
- Lumbar puncture

8.5 Assessments: MCI will be assessed using raters blinded to the study design. The cognitive battery will consist of: MoCA, CDR-SB and the Geriatric Depression Scale to help determine subject eligibility.

8.6 Safety Measurements:

a. Vital Signs - Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) will be measured while the patient is in sitting position after five minutes rest at each office visit.
b. Physical Exam – A physician will perform a physical exam on all MCI patients at the screening visit to evaluate for any significant medical conditions. Subsequent physical exams will be performed by an MD or Nurse Practitioner.

8.6.2 Other Assessments:

a. Demographic Data - Demographic data (gender, date of birth, marital status, ethnicity, race) will be collected for all patients.
b. Concomitant Medication - All meds taken by subjects as part of their routine health care will be documented during the study.

8.7 Participant Retention

Subjects who are enrolled in the study will be reimbursed for their time. Total study payment includes reimbursement for undergoing procedures and travel to the clinic for in-person study visits. Study partners who are required to accompany participants to their visits as well as monitor at home Flicker exposure sessions will also be reimbursed for their time.

Reimbursement will be made at the completion of the study. Emory University is required to complete form 1099 for any participant payments over $600 in a year. To comply with

this federal mandate the researchers are required to obtain subject's social security number to complete the form.

8.8 Schedule of Assessments
On the following pages, the schedule of assessments for both treatment groups is presented.

Table 1. Schedule of Assessments for the Non-Exposure Phase/Flicker Exposure Phase

Visit Number	1a	1b	2a	2b	3	4	5	6a[1]	6b	7	8	9	10	11a[2]	11b
Study Week	Screening[4]		Baseline[4]		1	2	3	4[4]	4[4]	5	6	7	8	9[4]	9[4]
Consent	X														
Confirm eligibility	X			X											
Medical history and medication review	X							X						X	
Physical exam and neurological exam	X			X				X						X	
Vital signs + height + weight	X			X											
Sensory function (see/hear)	X							X							
APOE genotyping		X													
Blood for biomarkers		X							X						X
Cognitive assessments	X			X				X						X	
Retinal imaging		X							X						X
MR imaging	X[5]		X[5]					X[3]	X[3]					X	
Lumbar puncture		X							X						X
PET Scan			X					X						X	
Eye tracking				X				X						X	
EEG evaluation and induced activity	X[6]			X				X						X	
Tolerance Testing	X							X						X	
Randomization/Study crossover				X				X							
Therapy training									X						
In-home observation visit										X					
In-home therapy										X	X	X	X		
Telephone call / email contact					X	X	X			X	X	X	X		X
Remote adherence monitoring										X	X	X	X		
Assessment of adverse events				X	X	X	X	X		X	X	X	X	X	

[1]Crossover visit (2-day visit)
[2]End of treatment visit (2-day visit)
[3]MRI will occur on either study visit 6a or 6b
[4]Study visits may extend over several days, depending on scheduling constraints
[5]MRI will be done at visit 1a if not done within the past 2 yrs or at 2a if done within the past 6 months
[6]Screening EEG may be repeated if entrainment criteria are not met or data are unreliable in subjects who have met all other eligibility criteria.

Table 2. Schedule of Assessments for the Flicker Exposure/Flicker Exposure Phase

Visit Number	1a	1b	2a	2b	3	4	5	6	7a[1]	7b	8	9	10	11a[2]	11b
Study Week	Screening[4]		Baseline[4]		1	2	3	4	5[4]	5[4]	6	7	8	9[4]	9[4]
Consent	X														
Confirm eligibility	X			X											
Medical history and medication review	X								X					X	
Physical exam and neurological exam	X			X					X					X	
Vital signs + height + weight	X			X											
Sensory function (see/hear)	X								X						
APOE genotyping		X													
Blood for biomarkers		X								X					X
Cognitive assessments	X			X					X					X	
Retinal imaging		X								X					X
MR imaging	X[5]		X[5]						X[3]	X[3]				X	
Lumbar puncture		X								X					X
PET Scan			X						X					X	
Eye tracking				X					X					X	
EEG evaluation and induced activity	X[6]			X					X					X	
Tolerance testing	X			X					X					X	
Randomization				X											
Therapy training				X											
In-home observation visit					X										
In-home therapy					X	X	X	X	X	X	X	X	X		X
Telephone call / email contact					X	X	X	X			X	X	X		
Remote adherence monitoring					X	X	X	X	X	X	X	X	X		
Assessment of adverse events				X	X	X	X	X	X		X	X	X	X	

[1]Midpoint clinic visit (2-day visit)
[2]End of treatment visit (2-day visit)
[3]MRI will occur on either study visit 7a or 7b
[4]Study visits may extend over several days, depending on scheduling constraints
[5]MRI will be done at visit 1a if not done within the past 2 yrs or at 2a if done within the past 6 months
[6]Screening EEG may be repeated if entrainment criteria are not met or data are unreliable in subjects who have met all other eligibility criteria.

9. DATA RECORDING, RETENTION, AND MONITORING

9.1 Electronic Case Report Forms

Electronic Case Report Forms will be completed for each patient who signs Informed Consent. All clinical information requested in this protocol will be recorded on the eCRFs. If an error is made on documents collected (source documents), a single line will be drawn through the error and the correct response will be written adjacent to the error; the change will be initialed and dated.

9.2 Retention and Availability Of Records

The Investigator will make study data accessible to Regulatory Agency inspectors upon request. A file for each patient will be maintained that includes the signed ICF and the Investigator's copies of all source documentation related to that patient. The Investigator will ensure the reliability and availability of source documents from which the information on the CRF was derived.

Study documentation will be maintained, including documents created or modified in electronic format, for at least 15 years following the completion of the study. ICFs, and adequate records for the receipt and disposition of all study drugs will be retained for a period of 2 years following the Food and Drug Administration (FDA) or other regulatory approval date of the drug or until 2 years after the drug investigational program is discontinued, unless a longer period is required by applicable law or regulation.

10. ASSAY MEASURES AND ANALYSES

10.1Genotyping for apolipoprotein E4 allele status will be performed in the Emory Genetics Core facility. Apolipoprotein E4 allele status will not be disclosed to study participants.

10.2 EEG. We will measure electrical brain activity using EEG from the scalp. For the EEG recording session, a cap (similar to a swim cap) will be placed on the participant's head and electrodes attached. Gel will be placed on the hair/head so that the electrodes can get a good connection to detect electrical charges that come from brain activity. This gel is easily removed with hair washing.

During the EEG study session, you will be seated in a chair. You will be asked to rest quietly with your eyes open. EEG will be recorded before, during and after use of the Flicker device (Figure 3). When the Flicker device is not in place, participants may be asked to perform simple movements, to perform a computer/iPad-based task with button presses, and/or to complete mood and cognitive assessment questionnaires.

There are no known risks of EEG. The gel in the hair can be sticky but is easily removed with shampooing. The EEG session may last up to 3 hours total time (including electrode placement and recording time) and will occur at 3 time points during the study: baseline, midpoint, and end-of-study.

EEG evaluation will be performed at Visits 1a and 2b for both groups to determine whether it is possible to entrain the gamma waves. If subjects' brain waves are unable to become entrained, they will be removed from the study. Screening EEG may be repeated if entrainment criteria are not met or data are unreliable in subjects who have met all other eligibility criteria.

10.3 Lumbar Puncture (LP): Lumbar puncture will be performed by a qualified clinician trained to perform the procedure. 20ml of CSF is collected using sterile technique and will be assayed for both known and novel biomarkers of disease, including inflammatory markers. Lumbar punctures will occur at the three in-person clinic visits (screening, midpoint, and end of study).

10.4 CSF and Serum Biomarkers. AD biomarkers including Aß42, tau, and phospho-tau levels in the CSF will be assayed at baseline, midpoint, and end of study visits. Specialty assays for inflammatory markers will also be conducted via a commercial vendor with specialized panels for measuring these markers (OLink, Sweden). Results are not disclosed to participants.

10.5 MRI. Brain MRI will be obtained or reviewed at screening to verify eligibility, at screening or baseline, at midpoint and end of study.

MRI films performed clinically within the past two years will be reviewed by the investigators for eligibility in the study. If a subject does not have clinical MRI films available, the MRI will be performed and reviewed prior to performance of the LP for safety reasons and to ensure that the subject meets eligibility criteria.

The 3T MR acquisition sequences will include all, or a subset of, the following 1) Structural MRI, 2) FLAIR,3) T2*GRE; 4) arterial spin labeling (ASL), and 5) resting state connectivity. MRI measurements of brain structure have been show to demonstrate brain atrophy (which correlates with neuron loss) in MCI and AD and increasing rates of brain atrophy as subjects become more impaired. Therefore, structural MRI is used as a measure of the rate of disease progression in AD treatment trials. Structural MRI (MPRAGE//IRSPGR) data will be used both as the rate of change as well as a predictor of future change. Cerebrovascular disease (especially white matter lesions (WMLs) will be assessed with FLAIR. Cerebral blood flow, which closely correlates with cerebral hypometabolism as an early biomarker of AD pathogenesis, will be measured by ASL imaging. Finally, resting state fMRI will be used to determine functional connectivity changes.

All MRIs will be reviewed by the investigators and the investigator will determine and document any clinically significant findings.

10.6 PET Scan. The PET scan includes injection of florbetapir (10 mCi [+/- 10%]) administered by intravenous bolus injection. During the 50-minute uptake phase, participants will wait in a quiet room. The PET scan will begin at approximately 50 minutes post-florbetapir injection. Brain images will be acquired continuously for a period of 20-40 minutes (scan time based on scan quality and the need for repeat sequences).

***10.7 Cognitive testing*.** Cognitive testing will occur at the baseline visit for each participant. A series of pen and paper or computer-based tests are administered to assess the current cognitive status (tests listed under Baseline visit 2b). At visit 1a, only the MoCA will be administered to determine eligibility.

***10.8 Study questionnaires*.** Interviews will be conducted at each of the three time points in the study: baseline, midpoint, and at end-of-study. These questionnaires are conducted with the study partner and are meant to assess subjective impressions of the participant's current behaviors and level of function. One questionnaire (the Clinical Dementia Rating [CDR]) also asks questions of the participant and helps to offer insight into their current beliefs about their cognitive status.

Statistical Analysis

Although this study is meant to assess tolerability and feasibility of Flicker exposure in the study population, there is a small chance that we will also obtain preliminary data that reflects clinical impact. However, this study is not powered to obtain generalizable results and the data will instead be used as pilot data for the preparation of a larger, early-phase clinical trial.

11. OPEN-LABEL EXTENSION

Participants who complete the clinical trial phase of the study will be eligible to participate in the open-label extension phase.

Subjects who elect to participate in the open-label phase will be asked to sign a new consent form (after the investigator or study coordinator has explained procedures for the open-label phase of the trial and after all participant questions have been addressed).

Participants who elect to participate in the extension phase will be required to come to the clinic at 6 and 12 months. Flicker administration will continue throughout these visits and be terminated only after Visit 15b. At 6 months (Visit 13), the following procedures will be performed:

- AE review
- Concomitant medication review

- Vital sign collection
- Physical and neurological exams
- CDR, NPI and FAQ

At 2 intervals between the clinic visits (Visits 12 and 14), the subjects will be contacted by telephone, during which time AEs and concomitant medications will be reviewed.

At Visit 15a and 15b, the following procedures will be performed:

- AE review
- Concomitant medication review
- Vital sign collection
- Physical and neurological exams
- Cognitive assessments – those performed in visit 11a
- MRI
- Amyloid PET scan (optional)
- Lumbar puncture (optional)
- EEG evaluation
- Tolerance testing
- Eye tracking
- Retinal imaging
- Blood draw

Subjects will be given the option to undergo the PET scan and the LP at Visit 15. They may elect to undergo both procedures or only one; they are required to undergo at least one of these procedures.

The EOS visit (Visit 16) will be conducted by telephone call, during which medications and AEs will be reviewed.

A schedule of assessments for the open-label phase of the study is found below:

Table 3. Schedule of Assessments for the Extension Phase

Visit Number	11a	11b	12	13	14	15a	15b	16
Study Week	Clinic 9[1]		Call 12(+/-2)[1]	Clinic 24(+/-2)[1]	Call 36(+/-2)[1]	Clinic 48(+/-2)[1]	Clinic 48(+/-2)[1]	Call Follow-up 52(+/-2)[1]
Consent (Confirm)	X							
Medical history and medication review	X		X	X	X	X		X
Physical exam and neurological exam	X			X		X		
Vital signs + height + weight	X			X		X		
Blood for biomarkers		X					X	
Cognitive assessments	X			X		X		
Retinal imaging		X					X	
MR imaging	X					X		
Lumbar puncture[2]		X					X	
PET Scan[2]	X					X		
Eye tracking	X					X		
EEG evaluation and induced activity	X					X		
Tolerance testing	X					X		
In-home therapy	X	X	X	X	X	X		
Telephone call / email contact			X		X			X
Remote adherence monitoring	X	X	X	X	X			
Assessment of adverse events			X	X	X	X		X

[1]Study visits may extend over several days, depending on scheduling constraints
[2]Subject may choose PET scan or lumbar puncture or both

Participants will be asked to report any adverse events that develop during the open-label phase of the trial.

The open-label phase of the study will be terminated after 1 year (i.e., 52 weeks) following successful completion of the active phase of the study. The P.I. reserves the right to stop the open-label phase of this study at any time.

12. POST-STUDY PERIOD

Participants will be allowed to retain the device for 1 year following the 1-year study period. Participants will not be required to come to Emory during this time to undergo any procedures.

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