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RESEARCH ARTICLE

A feasibility trial of gamma sensory flicker for patients with prodromal Alzheimer's disease

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Abstract

Introduction: We and collaborators discovered that flickering lights and sound at gamma frequency (40 Hz) reduce Alzheimer's disease (AD) pathology and alter immune cells and signaling in mice. To determine the feasibility of this intervention in humans we tested the safety, tolerability, and daily adherence to extended audiovisual gamma flicker stimulation.

Methods: Ten patients with mild cognitive impairment due to underlying AD received 1-hour daily gamma flicker using audiovisual stimulation for 4 or 8 weeks at home with a delayed start design.

Results: Gamma flicker was safe, tolerable, and adherable. Participants' neural activity entrained to stimulation. Magnetic resonance imaging and cerebral spinal fluid proteomics show preliminary evidence that prolonged flicker affects neural networks and immune factors in the nervous system.

Discussion: These findings show that prolonged gamma sensory flicker is safe, tolerable, and feasible with preliminary indications of immune and network effects, supporting further study of gamma stimulation in AD.

KEYWORDS
amyloid beta, cytokines, default mode network, electrophysiology, feasibility trial, gamma stimulation, neural stimulation, prodromal Alzheimer's disease, sensory stimulation

1 | INTRODUCTION

We and collaborators recently discovered that flickering light and sound at gamma frequency (specifically 40 Hz) drives gamma frequency neural activity in multiple brain areas and reduces amyloid pathology in mouse models of Alzheimer's disease (AD).[1–4] These changes coincide with transformed microglia, the primary immune cells of the brain, altered immune signaling, and improved cognitive functions.[1–5] These studies suggest a potential novel approach to treat AD, but the effects of gamma sensory flicker on AD pathology and

immune signaling in humans are unknown. Prior research has shown that sensory brain areas in humans entrain to flickering stimuli and that brain oscillations are stronger at the flickering frequency when stimulated for seconds to hours.[6–12] However it remains to be determined if extended gamma sensory stimulation, which may be required for AD treatment, is safe, tolerated, and feasible to perform daily over an extended period of time. Indeed, whether human subjects will reliably perform this stimulation for 1 hour per day for weeks or longer is a key question as the stimulation is not inherently rewarding. Thus, determining the safety, feasibility, and tolerability of this stimulation in AD patients is required to advance this new therapeutic approach to AD. Accordingly, we performed a feasibility study in a small cohort of human participants to test the safety of extended gamma flicker stimulation, tolerance to this stimulation, and adherence during home use in patients with amnestic mild cognitive impairment (MCI) and confirmed AD biomarkers (i.e., prodromal AD). We assessed target engagement of 40 Hz neural activity during sensory flicker. We also explored preliminary biological effects to inform future trials. Because the beneficial effects of gamma stimulation likely arise from engaging plasticity and immune mechanisms, we measured changes in neural activity, neural circuits, and immune signaling in addition to markers of AD pathology (amyloid beta [$A\beta_{42}$], total tau [t-tau], and phosphorylated tau [p-tau]).

In this study, 10 individuals with prodromal AD received 1-hour daily combined visual and auditory gamma flicker for 4 or 8 weeks. We targeted MCI patients because these participants would be best able to express the tolerability of the stimulation. Using a delayed start design, we assessed the effects of 4 weeks of no stimulation (e.g., effects related to disease progression or repeated measurements), 4 weeks of stimulation, and 8 weeks of stimulation. While we expect future studies and therapeutic use to extend for months or years, we anticipated that 4 to 8 weeks of stimulation would be long enough to assess safety and adherence and show some preliminary biological effects. Herein we report on safety, tolerability, adherence, neural activity, AD pathology ($A\beta_{42}$, t-tau, and p-tau), and immune signaling from a pilot trial of 4 to 8 weeks of gamma flicker stimulation.

2 | MATERIALS AND METHODS

2.1 | Study design

This delayed-start trial was conducted at the Emory Brain Health Center and was approved and monitored by the Institutional Review Board at Emory University and by an independent clinical research analyst. This study was an investigator initiated study sponsored by Cognito Therapeutics, Inc. and is documented on clinicaltrials.gov under NCT03543878. Participants were randomized to receive flicker exposure (via a light and sound device-based stimulation) for either 8 weeks or 4 weeks under a delayed-start protocol with weekly telephone check-ins (Figure S1 in supporting information, Figure 1A). We did not use a sham stimulus for comparison because flickering stimuli in other patterns had different effects than no stimulation in the

HIGHLIGHTS

- We tested the feasibility of audiovisual gamma flicker in individuals with mild cognitive impairment.
- Gamma flicker was safe, tolerable, and feasible to perform at home for 4 to 8 weeks.
- Gamma flicker results in increased gamma neural activity during stimulation.
- Preliminary evidence suggests that flicker strengthened functional connectivity.
- Preliminary evidence suggests flicker altered brain immune factors.
- Our findings support further study of gamma stimulation in Alzheimer's disease.

RESEARCH IN CONTEXT

1. **Systematic Review**: The authors performed a literature search using PubMed and Google Scholar to identify relevant publications and meeting abstracts. The most relevant papers identified are appropriately cited.

2. **Interpretation**: These searches showed that gamma sensory stimulation reduced Alzheimer's disease (AD) pathology, altered immune signals, and rescued spatial memory behavior in animal models. Studies showed that sensory stimulation entrains neural activity in humans; however, the effects of prolonged gamma sensory stimulation in humans are unknown. A trial was designed to test the feasibility of prolonged gamma stimulation in human participants with prodromal AD.

3. **Future directions**: This work supports the safety, tolerability, and feasibility of gamma sensory stimulation over weeks in prodromal AD patients. Participants entrained to stimulation and preliminary biological effects on neural activity, neural networks, and immune signals were identified. This research is an essential step to initiate a larger, longer, sham-stimulation-controlled trial of this novel therapeutic approach.

5XFAD mouse model of AD and in wild-type mice.[1,5] Participants underwent venous blood draws, lumbar punctures, magnetic resonance imaging (MRI) scans, electroencephalograms (EEGs), and cognitive testing at baseline (see Supplementary Methods in supporting information for details). All procedures except for cognitive testing were repeated at midpoint and at the end of study. Lumbar punctures performed within the prior 6 months were used for baseline when available.
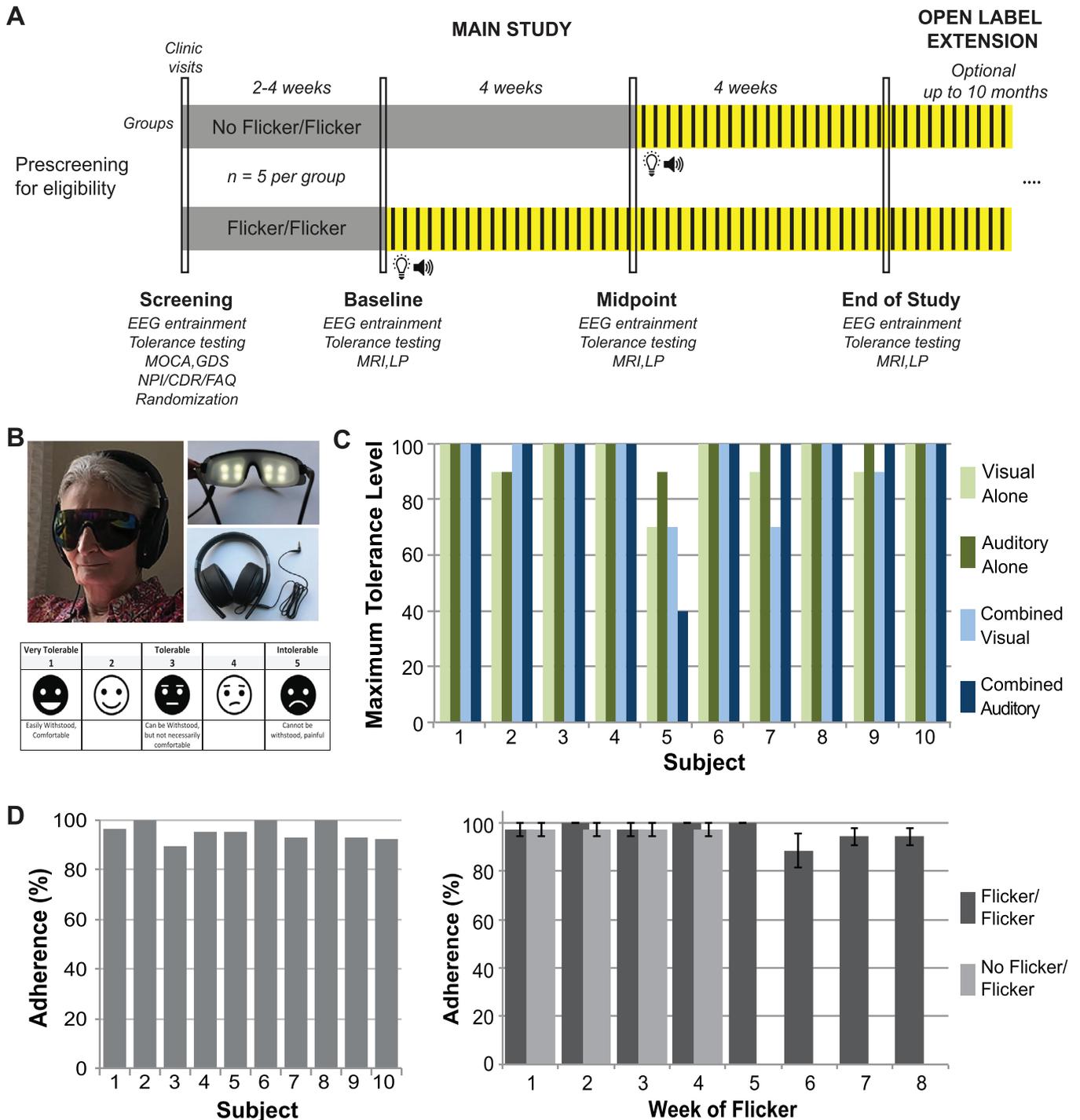
FIGURE 1 Tolerance and adherence to gamma sensory flicker stimulation. A, Overview of study design. Horizontal gray bars indicate no stimulation, horizontal yellow and black striped bars indicate when subjects were instructed to perform 1 hour/day gamma sensory flicker, vertical bars indicate clinic visits with tests performed described below the bars. B, Gamma sensory stimulation device (top left) consists of a pair of light-emitting goggles (top right) and a pair of sound-emitting headphones (middle right) that turned on and off with a repetition rate of 40 Hz. Subjects rated their tolerance to flicker stimulation using a tolerance testing scale (bottom). C, Maximum tolerated stimulation levels (in percentage of maximum) for visual alone, auditory alone, and visual or auditory flicker when combined for each subject at baseline. D, Adherence rates per subject over 4 to 8 weeks of 1 hour/day flicker treatment (left) and adherence rates per week and for the flicker/flicker (dark gray) and no flicker/flicker (light gray) groups (right)

2.2 | Participants

Target enrollment was 10 participants with MCI due to AD, as evidenced by positive cerebrospinal fluid (CSF) biomarkers of AD. Participants were enrolled at Emory University from October 2018 through October 2019. Participants were recruited from the Emory Cognitive Neurology Clinic and the Emory Goizueta Alzheimer's Disease Research Center (ADRC). Diagnosis was made by expert clinicians from the ADRC and included CSF biomarker testing to confirm etiological diagnosis (see Supplementary Methods for all inclusion and exclusion criteria). Sample size was determined to assess tolerability and adherence in line with previous Phase 1 trials.[13–16] Written informed consent was obtained from each participant by study staff.

2.3 | Outcomes

The primary outcomes of this pilot study were safety, tolerability, and feasibility of once-daily, 1-hour gamma sensory flicker exposure. These measures were assessed by the number of reported adverse events, compliance, and routine clinical and laboratory assessments (i.e., vital signs and physical exams). Tolerability was defined by the participants' ability to withstand stimulation in a tolerance assessment (See Tolerance Testing in supporting information). Feasibility was defined as adherence to daily flicker exposure for 1 hour at home. Exploratory outcomes included: immune factors, $A\beta_{42}$, t-tau, and p-tau changes in CSF; alterations in brain functional connectivity measured using resting state function MRI (fMRI), and neural activity measured using EEG. Persons performing data analysis were blind to group assignment.

2.4 | Statistical analysis

Paired t-tests were performed to compare differences before and after 4 weeks of no flicker, 4 weeks of daily flicker, and 8 weeks of daily flicker. These differences included number of entrained channels (EEG), percent of power at 40 Hz (EEG), percent of power (normalized power from 1 to 50 Hz; EEG), functional connectivity (fMRI), CSF AD biomarkers, and CSF Luminex/Olink inflammation panels. P values were false discovery rate (FDR, and indicated p_{FDR} for P values) corrected in the EEG analysis for the number of frequency bands (e.g., delta, theta, alpha, beta, gamma) compared within a flicker exposure duration. For comparisons that were made over many MRI voxels or EEG channels, we did not report mean and confidence intervals on non-significant results due to the high number of comparisons.

3 | RESULTS

3.1 | Baseline participant characteristics

No differences in general characteristics between groups that received 8 weeks of daily flicker (flicker/flicker) or 4 weeks of no flicker and 4 weeks of daily flicker (no flicker/flicker, Table 1) were noted. All participants had high educational status and a committed study partner, usually a spouse. One participant had a much higher Neuropsychiatric Inventory than others (69 versus an average of 8.3 for others) and was observed to be more agitated than other participants.

3.2 | Tolerance

Participants rated flicker stimulation tolerability for different levels of light and sound intensity. Enrolled participants found a wide range of stimulation levels tolerable and nine participants' maximum tolerable level was greater than 70% intensity (Figure 1B,C). Of 17 participants screened for the study, 1 participant was excluded from the study because the flicker stimulation was not tolerated. Another screened participant was excluded because pre-existing tinnitus worsened after flicker tolerance testing. An additional indication of tolerability was whether participants opted to continue using flicker stimulation after the main study was completed. Participants were given the option to enroll in an open label extension at study completion. Nine participants enrolled in the optional open label extension choosing to continue daily flicker stimulation for up to 1 year. Three participants later dropped out of the open label extension, two due to unrelated health changes and one due to headaches that were likely flicker related. Thus, 15/17 (88%) screened participants found flicker stimulation tolerable and 9/10 (90%) of enrolled participants initially continued flicker stimulation in an optional study extension, one of which later dropped out due to flicker-related problems.

3.3 | Adherence

Participants adhered well to flicker therapy over 4 to 8 weeks as measured by the percentage of days they used the device out of the total days they were assigned to use it. Adherence rates during the main phase of the study were 95.5% on average with all participants having adherence rates greater than 89% during the main phase of the study (Table S1 in supporting information, Figure 1D). Adherence rates per week remained above 88% on average over 8 weeks (Table S2 in supporting information, Figure 1D).

3.4 | Safety

Participants experienced no severe adverse events related to flicker (Table 2). Adverse events possibly associated with flicker treatment were mild and included dizziness, tinnitus, headache, and worsened hearing loss (Table 2 includes complete list of unrelated adverse events). Adverse events were tracked during the main study (4–8 weeks of flicker per patient) and the open label extension, which was ongoing at the time of this report (includes more than 20 weeks of flicker per patient).

TABLE 1 Baseline characteristics of the study participants

| Characteristics | Subject | | | | | | | | | | Mean & SD per group | | | |
	1	2	3	4	5	6	7	8	9	10	F/F		N/F	
Age	61	77	63	71	75	80	78	65	66	77	72	8.15	70.6	6.54
Sex	M	F	F	M	M	M	F	M	F	F				
Education (y)	16	16	16	16	16	20	20	20	18	18	18	2.00	17.2	1.79
MCI onset	2014	2018	2018	2018	2017	2016	2011	2019	2017	2014	2015	2.55	2017	1.95
Marital status	M	M	M	M	M	M	M	M	M	W				
Language	Eng	Eng	Eng	Eng	Eng	Eng	Eng	Eng	Eng	Eng				
MoCA	17	29	21	15	26	22	21	18	22	21	21.6	3.21	20.8	5.22
GDS	10[a]	5	1	2	2	2	2	1	1	0	1.75	0.50	1.8	1.92
CDR	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.00	0.5	0.00
NPI	6	16	2	4	69[b]	9	5	16	12	5	8	3.16	8.6	6.84
FAQ	14	3	4	1	6	5	16	11	11	12	10.4	4.83	6.2	4.97
Group	F/F	N/F	N/F	N/F	F/F	F/F	F/F	N/F	F/F	N/F				

Abbreviations: CDR, Clinical Dementia Rating; Eng, English; F/F, flicker/flicker group; FAQ, Functional Assessment Questionnaire; GDS, Geriatric Depression Scale; M, married; MCI, mild cognitive impairment; MoCA, Montreal Cognitive Assessment; N/F, no flicker/flicker group; NPI, Neuropsychiatric Inventory; SD, standard deviation; W, widowed.

[a]Beck Depression Inventory not GDS was administered for baseline for this participant due to age. This value is excluded from mean and standard deviation.
[b]This NPI was excluded from the summary statistics because it is an outlier.

TABLE 2 Summary of adverse events

| Adverse event | Number of events | | Subjects affected | Related to treatment |
	Main	OLE		
Dizziness	3	0	S1, 10	Possibly
Tinnitus or "buzzing in ears"	1	1	S1, 5	Possibly
Headache	1	2	S1, 7	Possibly; probably
Double vision	1	0	8	No
Worsened hearing loss	0	1	5	Possibly
Cataracts and surgical removal	0	2	5	No
Leg, arm, joint pain	5	0	5, 10	No
Back pain	3	1	5, S2, 7	No
Depression	1	0	5	No
Rhinorrhea	1	2	1, 5, 9	No
Gastrointestinal problems	1	1	5, 7	No
Fall	1	0	4	No
Dog bite	1	0	10	No
Skin growth on neck	1	0	7	No
Prolapsed uterus	0	1	7	No
Hemorrhoids	1	0	7	No

Abbreviations: Main, main study; No., number; OLE, open label extension; S, screened subject that was not enrolled.

3.5 | EEG entrainment to flicker

All screened and enrolled participants entrained to 40 Hz audiovisual flicker during each study visit, indicating target engagement of 40 Hz neural activity. On average, 49 out of 64 channels were entrained (Figure 2A). Entrainment to flicker during screening was required for study participation. To determine whether EEG activity changed after daily flicker, we compared EEG recordings before (pre) and after (post) 4 weeks of no flicker (flicker), 4 weeks of flicker, and 8 weeks of flicker. No significant differences in the number of channels entrained were found
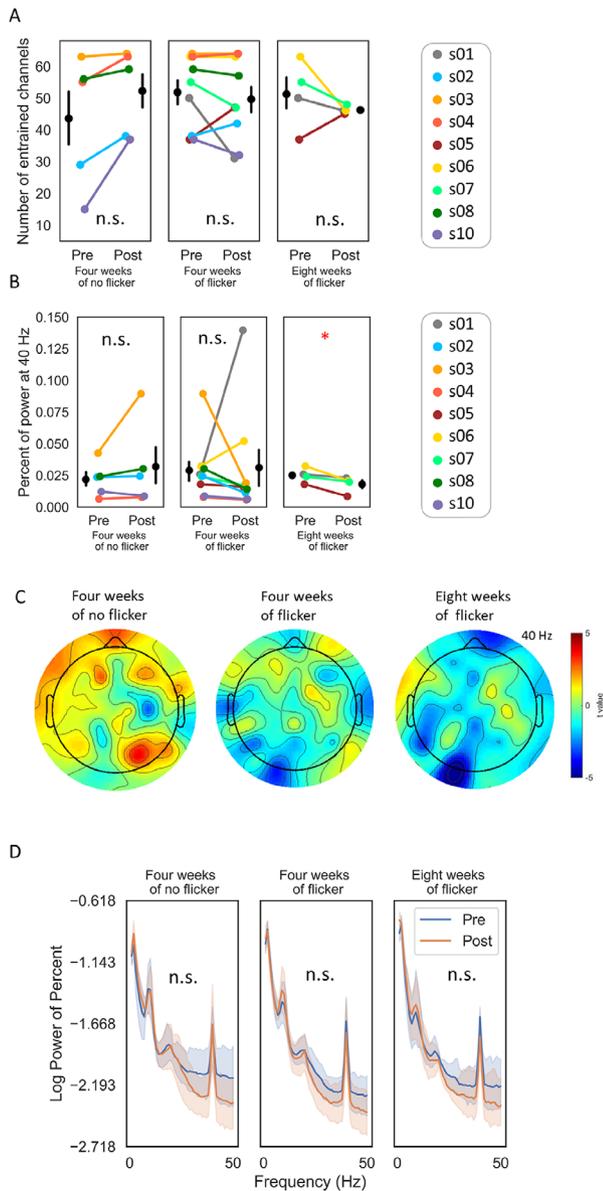
FIGURE 2 Gamma entrainment during audiovisual flicker as a function of flicker exposure duration. A, Number of channels entrained during flicker exposure before (pre) and after (post) 4 weeks of no flicker (left), 4 weeks of daily flicker (middle), and 8 weeks of daily flicker treatment (right). Each dot is a different subject. Black lines and error bars on the side indicate mean ± standard error of the mean. B, Percent of power at 40 Hz averaged across all channels during flicker exposure pre and post 4 weeks of no flicker (left), 4 weeks of daily flicker (middle), and 8 weeks of daily flicker treatment (right). Each dot is a different subject. Black lines and error bars on the side indicate mean ± standard error of the mean. C, Change in percent power at 40 Hz as a function of flicker exposure across channels for 4 weeks of no flicker (left), 4 weeks of daily flicker (middle), and 8 weeks of daily flicker treatment (right). Color map indicates t value of pair-wise comparisons with colder colors indicating power percent is weaker in the post-flicker visit than in the pre-flicker visit. D, Log transformation of percent of power across frequencies from 1 to 50 Hz during flicker exposure pre (blue) and post (orange) 4 weeks of no flicker (left), 4 weeks of daily flicker (middle), and 8 weeks of daily flicker treatment (right). Solid lines indicate mean, shaded area indicates standard error of the mean. n.s. not significant, * $P < 0.05$

in any of the pre versus post (pre–post) comparisons (P-values > 0.11) in any flicker exposure group (Figure 2A).

We next asked whether the percent of power at 40 Hz (i.e., the raw power at 40 Hz divided by the sum of power from 1 to 50 Hz; see Methods) changed as a function of flicker exposure duration, averaged across all 64 channels. Neither the 4-week no flicker nor the 4-week flicker groups showed significant differences in the pre–post comparisons (P-values > 0.32, Figure 2B). However, after 8 weeks of daily flicker, there was a small but significance reduction in power at 40 Hz during flicker (paired t-test; 0.7%, 95% confidence interval [CI]: 0.12% to 1.3%, $P = 0.04$; Figure 2B; similar trends were seen for absolute power, Figure S2A in supporting information). When analyzed per channel, we observed the strongest decreases in the P7 and O1 channels located in the left occipital lobe after 8 weeks of daily flicker (P7: –1.09%, 95% CI: –2.09% to –0.001%, $p_{FDR} = 0.21$; O1: –0.68%, 95% CI: –1.00% to –0.37%, $p_{FDR} = 0.01$; uncorrected P-values < 0.05; Figure 2C). We did not find any significant changes in percent of power in other frequency bands (delta: 1–3 Hz; theta: 4–7 Hz; alpha: 8–12 Hz; beta: 13–30 Hz; gamma: 3–50 Hz; Figure 2D, FDR correction applied; absolute power in Figure S2A). In sum, 40 Hz entrainment was sustained across participants. In addition, 40 Hz power during flicker stimulation slightly decreased in the left occipital region after 8 weeks of daily flicker.

3.6 | Increased default mode network functional connectivity after 8 weeks of daily flicker

During resting state, functional connectivity in the default mode network is lower in AD patients compared to healthy controls.[17–22] Therefore, to assess flicker's effects on resting state and regional connectivity relevant to AD, we assessed functional connectivity using fMRI between posterior cingulate cortex (PCC) and precuneus (PCu) and between PCC and medial prefrontal cortex (mPFC) nodes of the default mode network (Figure 3A). Functional connectivity between PCC and PCu significantly increased after 8 weeks of daily flicker (Figure 3B, paired t-test; 0.181, 95% CI: 0.092–0.269, $P = 0.016$) but not in other groups (P-values > 0.25). No significant differences were found in the functional connectivity between PCC and mPFC across any exposure period (Figure 3C, P-values > 0.13). In sum, the functional connectivity between PCC and PCu, which is weakened in AD, was strengthened after 8 weeks of daily flicker.

3.7 | No significant changes in CSF Aβ and tau levels after 4 or 8 weeks of daily flicker

To assess potential changes in AD biomarkers, levels of Aβ_{42}, p-tau, t-tau, and the ratio t-tau/Aβ_{42} were assessed in the CSF of participants before and after 4 weeks of no flicker, 4 weeks of daily flicker, and 8 weeks of daily flicker. We found no significant differences in the changes in Aβ_{42}, p-tau, t-tau, or t-tau/Aβ_{42} ratio after 4 or 8 weeks of daily flicker ($P > 0.26$, paired T-tests, Figure S3 in supporting information).

A

Precuneus (PCu)
Posterior cingulate cortex (PCC)
medial prefrontal cortex (mPFC)

B

C
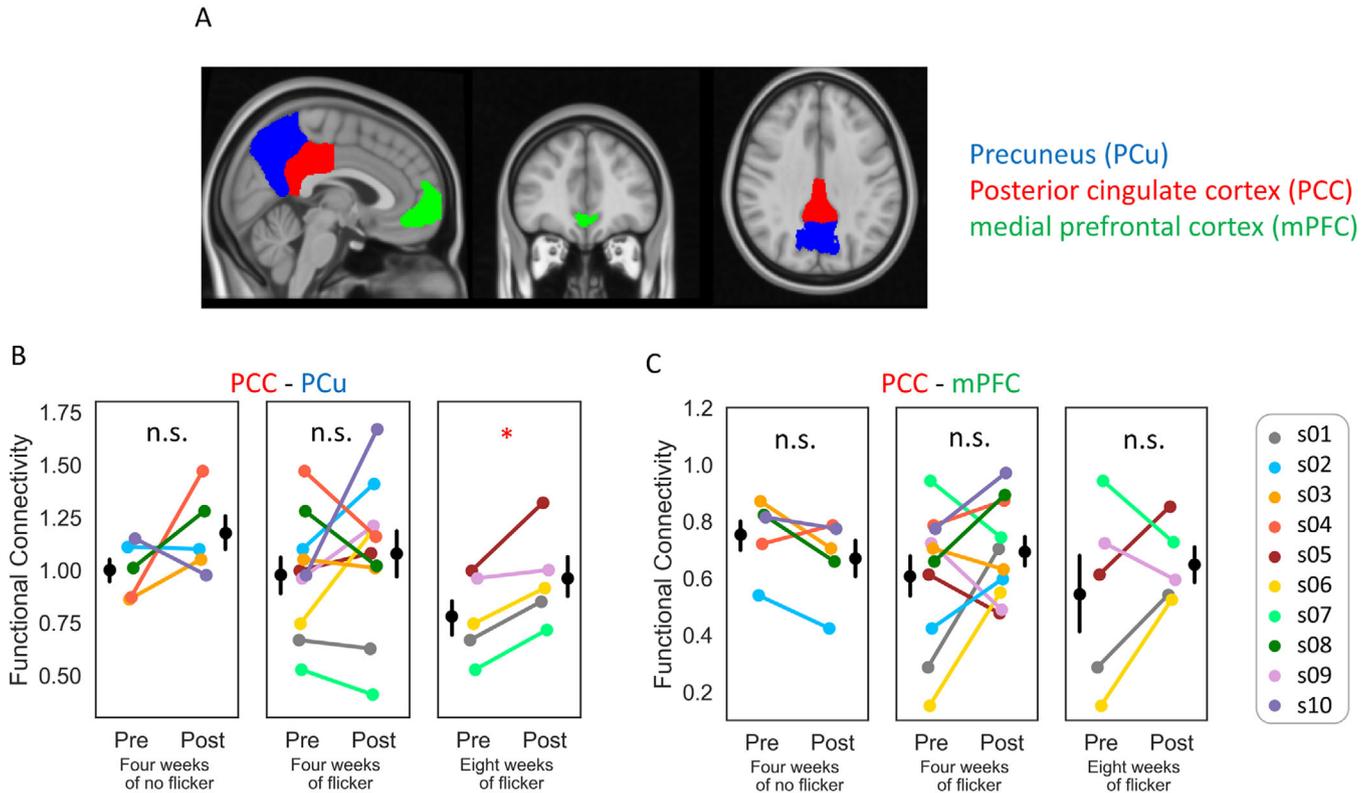
FIGURE 3 Resting state functional connectivity changes as a function of flicker exposure duration. A, Regions of interest were precuneus (PCu, blue), posterior cingulate cortex (PCC, red), and medial prefrontal cortex (mPFC, green). B, Functional connectivity between PCC and PCu pre and post 4 weeks of no flicker (left), 4 weeks of daily flicker (middle), and 8 weeks of daily flicker treatment (right). C, As in (B) for functional connectivity between PCC and mPFC. Black lines and error bars on the side indicate mean ± standard error of the mean. n.s. not significant, * $P < 0.05$
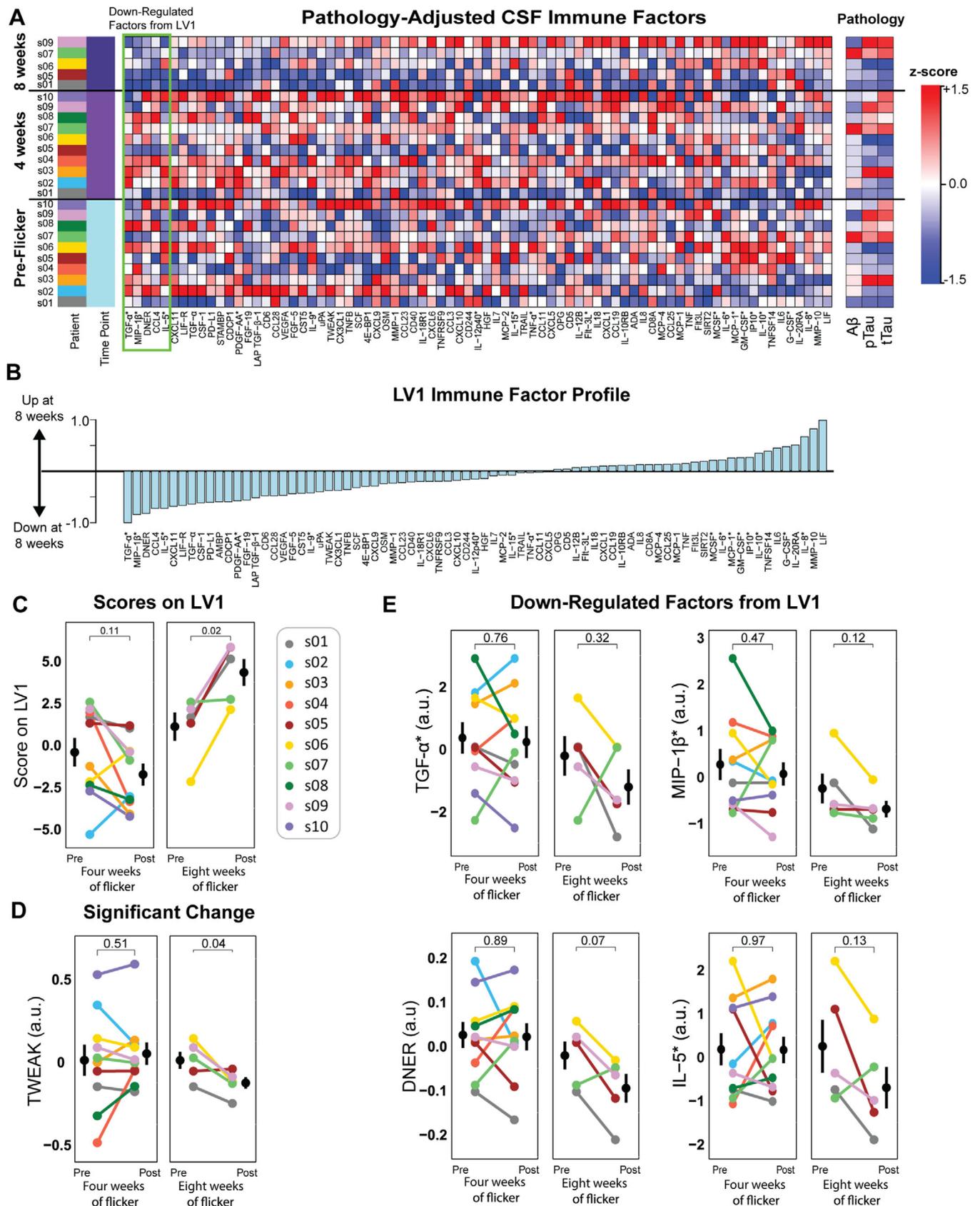
3.8 | Altered levels of cytokines and immune factors in CSF after 8 weeks of daily flicker

Having previously found that 40Hz flicker stimulates microglial activation and increases cytokine expression in mice,[1,3,5] we asked if flicker alters levels of cytokines and other immune factors as evidence of immune engagement in humans. To isolate the effects of flicker on immune factors regardless of differences in individuals' underlying $A\beta_{42}$ and p-tau pathology, we adjusted for effects related to $A\beta_{42}$ and p-tau using a linear model (Figure 4A, Figure S4 in supporting information). We then used a partial least squares discriminant analysis (PLSDA, see Supplementary Methods) to identify a latent variable 1 (LV1), that distinguished among pre-flicker, 4-week, or 8-week flicker groups (Figure 4B). LV1 was made up of a weighted profile of cytokines and immune factors, with negative values indicating a decrease in that cytokine after 8 weeks of flicker (Figure 4B). Using paired t-tests comparing samples from the same participant at different time points, LV1 was unchanged after 4 weeks of flicker (−1.32, $P = 0.11$) but increased after 8 weeks compared to pre-flicker (3.23, $P = 0.02$; Figure 4C). The cytokine TWEAK (tumor necrosis factor-related weak inducer of apoptosis) significantly decreased after 8 weeks of flicker (−0.14, $P = 0.04$; Figure 4D). Several factors showed trends of downregulation after 8 weeks of flicker including transforming growth factor alpha (TGF-α),

macrophage inflammatory protein 1β (MIP-1β), Delta and Notch-like epidermal growth factor receptor (DNER), and interleukin (IL)-5 (Figure 4E). In sum, changes in the immune profile in CSF showed trends toward downregulation of immune factors suggesting engagement of the neuroimmune system after chronic exposure to audiovisual flicker.

4 | DISCUSSION

As an initial step in exploring audiovisual gamma sensory stimulation as a potential therapeutic approach to AD, we performed a feasibility study in a small cohort of human participants with prodromal AD to assess safety, tolerance to extended daily stimulation, and adherence during home use. We assessed neural entrainment, CSF $A\beta_{42}$ and tau proteins, default mode network functional connectivity, and immune factors to establish evidence for target engagement and preliminary indications of potential therapeutic mechanisms. We found gamma flicker was safe with no serious adverse events related to treatment. Sixteen of 17 screened participants found the stimulation tolerable, and adherence rates during the main phase of the study were excellent (95.5% on average). All participants entrained to gamma stimulation at all time points assessed. While the study was not powered to

FIGURE 4 Cytokines and immune factors are altered after 8 weeks of flicker. A, Cerebrospinal fluid (CSF) immune factor data corrected for amyloid beta $(A\beta)_{42}$ and phosphorylated tau. Each row is one subject at one time point and each column is one cytokine, immune factor, or Alzheimer's disease (AD) pathology biomarker. * indicate factors measured via Luminex while other factors were measured via Olink. Green box indicates most downregulated factors from latent variable 1 (LV1) in (B) and (E). B, The weighted profile of immune factors in latent variable 1 (LV1

draw conclusions about biological changes after relatively short treatment periods, we found preliminary evidence that flicker strengthened functional connectivity in the default mode network and altered cytokines and immune factors in the CSF. The findings from this pilot study support viability of testing long-term multisensory gamma frequency stimulation as a potential therapeutic approach to AD. Further testing with a larger sample size; longer duration of treatment; and a blinded, placebo-controlled design are warranted to test the potential benefit of this novel therapeutic approach for patients with AD.

Our current findings strongly support safety and feasibility of chronic, daily flicker as a potential treatment for patients with mild symptoms of AD. Participants were very compliant during the main study period, and 90% elected to continue using flicker for up to a year in an open label extension. The low rate of adverse events in this study was quite reassuring. However, we excluded individuals with a history of migraines, tinnitus, or seizures because sensory stimuli can potentially exacerbate these conditions. Seizures are a potential risk even in those without a prior history, but the rate of sensory-triggered seizures in the general population is very low ($\approx$1 per 10,000).[23] One participant with a history of tinnitus was screened because the participant and their physician thought this tinnitus history would not be a problem. However, the tinnitus got worse after sensory stimulation and the participant did not continue with the study.

This study builds on prior research in mouse models of AD in which we and collaborators found that stimulating gamma frequency neural activity reduced amyloid pathology and recruited microglia, the primary immune cells in the brain.[1–4] Gamma activity has long been theorized to facilitate neural communication but was not previously suspected to play a role in immune function. Gamma flicker also increased proteins that support plasticity and prevent synaptic loss.[1,4] Based on these studies, we expect driving gamma frequency neural activity could be therapeutic in AD by altering immune signaling and by stimulating or preserving plasticity in neural networks.

Chronic flicker strengthened functional connectivity between nodes in the default mode network, particularly between PCC and PCu, which are weaker in AD compared to healthy controls.[17–22] In addition, a prior small study showed PCC–PCu functional connection strength was positively correlated with cognitive function.[24] Evidence that PCC–PCu connectivity was strengthened after chronic flicker suggests a change toward normal function. The small decrease in gamma power observed with flicker was unexpected, and we speculate that the finding could reflect a homeostatic response to repeated stimulation and gamma entrainment.[25] Evidence for altered immune factors and cytokines in the CSF after 8 weeks of daily flicker strongly supports engagement of the neuroimmune system. We found trending downregulation of immune factors that stimulate astrocyte (TGF-α[26]

and microglial proliferation (IL-5[27]) and microglial motility (MIP-1β[28]). We found significant downregulation of TWEAK, which regulates key immune signaling cascades including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling, cytokine expression, and matrix metalloproteinase production.[29] Moreover, inhibition of TWEAK has shown therapeutic potential in several animal models of neurological diseases.[29] Together, these data suggest that long-term flicker therapy may attenuate potentially harmful cytokines involved in activation of microglia and astrocytes.

This study had several limitations, including its small size and short duration. Furthermore, the study did not include a placebo condition because results from animal studies indicated that sham stimuli might have unintended effects. In mice, randomized stimulation (where the inter-stimulus interval was randomized) increased Aβ levels,[1] and we found different types of visual stimulation have distinct effects on cytokines and extracellular signals.[5] It is unclear what stimulus patterns might be used as an appropriate placebo control for audiovisual flicker. Despite these limitations, the results of this pilot study support the feasibility of pursuing gamma audiovisual stimulation as a novel, non-invasive, non-pharmacological approach to treating individuals with AD.

The results of this study address potential concerns about participant safety, adherence, and tolerance to daily home use of gamma sensory stimulation and provide insights for how to maintain adherence in future studies. Furthermore, we found strong target engagement in that all participants significantly entrained to 40 Hz flicker. The primary limitations of this study, the small size and a lack of a sham stimulus, must be addressed in future studies. Possible clinical benefits from gamma sensory stimulation would likely arise from flicker inducing long-term changes in neural activity, neural circuits, and immune signaling. We found preliminary such effects on neural activity via resting state EEG, on neural circuits via default mode connectivity in fMRI, and on immune signaling via changes in cytokines and immune factors in the CSF. Together these results show chronic flicker is feasible in MCI participants and reveal preliminary biological effects in humans, supporting the case for further study of this novel approach to treat AD.

ACKNOWLEDGMENTS

immune factor profile) that distinguished pre-flicker (pre) from after 8 weeks of daily flicker (post) ordered from factors that were most downregulated (negative weights) to those that were most upregulated (positive weights) after 8 weeks of flicker. C, LV1 scores for each participant pre and post 4 (left) or 8 (right) weeks of flicker (paired t-tests). D, TWEAK (tumor necrosis factor-related weak inducer of apoptosis) pre and post 4 (left) or 8 (right) weeks of flicker (paired t-test). E, The most downregulated factors from LV1 from the left side of (C) pre and post 4 (left) or 8 (right) weeks of flicker (paired t-tests). Note that macrophage inflammatory protein 1β (MIP-1β*) and CCL4 are the same cytokine and MIP-1β* was selected to display. Black bars in (C), (D), and (E) are mean $\pm$ standard error of the mean

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AUTHOR CONTRIBUTIONS

James J. Lah had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Annabelle C. Singer, James J. Lah, Allan I. Levey, Margaret L. Walker, Cecelia M. Manzanares designed the study; Kay M. Colon-Motas, Lydia Piendel, Jonna K. Seppa, Margaret L. Walker acquired data; Qiliang He, Alyssa F. Pybus, Levi B. Wood analyzed data; Levi B. Wood, Svjetlana Miocinovic, Deqiang Qiu, Allan I. Levey, James J. Lah, Annabelle C. Singer interpreted results; Annabelle C. Singer, Levi B. Wood, Qiliang He, Kay M. Colon-Motas, Lydia Piendel, Alyssa F. Pybus, Jonna K. Seppa drafted the manuscript. All authors provided critical feedback and edited the manuscript. James J. Lah and Annabelle C. Singer supervised all aspects of the study.

CONFLICTS OF INTEREST

Annabelle C. Singer owns shares in Cognito Therapeutics, which aims to develop gamma stimulation-related products; these conflicts of interest have been disclosed to and are managed by the Georgia Institute of Technology Office of Research Integrity Assurance. Since the completion of this study, Dr. Levey serves as a consultant to Cognito Therapeutics, who provides funding support for this study and manufactures products used in this study. Dr. Levey receives compensation for these services. The terms of this arrangement have been reviewed and approved by Emory University in accordance with its conflict of interest policies. The authors declare no other competing financial interests.

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