bottNEURO
precision medicine for brain health

Non-invasive electrical stimulation for the treatment of Alzheimer's disease CLINICAL TRIALS

www.bottneuro.com

Non-invasive electrical stimulation for the treatment of Alzheimer's disease CLINICAL TRIALS

STUDY	CONCLUSIONS
Tele-supervised home-based transcranial alternating current stimulation (tACS) for Alzheimer's disease: a pilot study (2023) Davide Cappon, Rachel Fox, Tim den Boer, Wanting Yu, Nicole LaGanke, Gabriele Cattaneo, Ruben Perellón-Alfonso, David Bartrés-Faz, Brad Manor, Alvaro Pascual-Leone Harvard Medical School, Boston, USA	This study highlights the importance of targeted 40 Hz tACS to improve specific cognitive functions and demonstrates the feasibility and safety of long-term, home-based, remotely supervised 40 Hz tACS Alzheimer's disease therapies. Over the course of 9 months, patients diagnosed with Alzheimer's disease received repeated 40 Hz tACS. In all patients, an improvement in episodic memory, associated with the stimulated brain region, was detected.
Impact of 40 Hz Transcranial Alternating Current Stimulation on Cerebral Tau Burden in Patients with Alzheimer's Disease: A Case Series (2022) Maeva Dhaynaut, Giulia Sprugnoli, Davide Cappon, Joanna Macone, Justin S. Sanchez, Marc D. Normandin, Nicolas J. Guehl, Giacomo Koch, Rachel Paciorek, Ann Connor, Daniel Press, Keith Johnson, Alvaro Pascual-Leone, Georges El Fakhri, Emiliano Santarnecchi Harvard Medical School, Boston, USA	This case study provides preliminary evidence for the disease-modifying properties of repeated 40 Hz tACS in Alzheimer's disease by inducing protein clearance and modulating neuroinflammation. After 4 weeks of treatment, a reduction in pTau and microglia activity was observed.
Increasing Brain Gamma Activity Improves Episodic Memory and Restores Cholinergic Dysfunction in Alzheimer's Disease (2022) Alberto Benussi, Valentina Cantoni, Mario Grassi, Lucie Bréchet, Christoph M. Michel, Abhishek Datta, Chris Thomas, Stefano Gazzina, Maria Sofia Cotelli, Marta Bianchi, Enrico Premi, Yasmine Gadola, Maria Cotelli, Marta Pengo, Federica Perrone, Maria Scolaro, Silvana Archetti, Eino Solje, Alessandro Padovani, Alvaro Pascual-Leone, Barbara Borroni University of Brescia, Brescia, Italy	This randomized, double-blind clinical trial presents the beneficial effects of a single session of 40 Hz tACS on cognitive functions in Alzheimer's disease patients. By stimulating specific cortical nodes, neural activity and episodic memory functions are improved. Additionally, the observed increase in cholinergic transmission indicates a restoration of intracortical excitability.
Impact of multisession 40 Hz tACS on hippocampal perfusion in patients with Alzheimer's disease (2021) Giulia Sprugnoli, Fanny Munsch, Davide Cappon, Rachel Paciorek, Joanna Macone, Ann Connor, Georges El Fakhri, Ricardo Salvador, Giulio Ruffini, Kevin Donohoe, Mouhsin M. Shafi, Daniel Press, David C. Alsop, Alvaro Pascual Leone & Emiliano Santarnecchi Harvard Medical School, Boston, USA	In this study, the beneficial biological effects of repeated 40 Hz tACS for the treatment of Alzheimer's disease are demonstrated. Within 4 weeks of treatment, an increase in fast brain oscillations and cerebral blood perfusion was observed in patients with mild to moderate Alzheimer's disease.

MIAMIND

Non-invasive electrical stimulation for the treatment of Alzheimer's disease
CLINICAL TRIALS

STUDY	CONCLUSIONS
Patient-Tailored, Home-Based Non-invasive Brain Stimulation for Memory Deficits in Dementia Due to Alzheimer's Disease (2021) Lucie Bréchet, Wanting Yu, Maria Chiara Biagi, Giulio Ruffini, Margaret Gagnon, Brad Manor, Alvaro Pascual-Leone Harvard Medical School, Boston, USA	This pilot study demonstrates the safety and feasibility of repeated, personalized home-based tACS targeting the left angular gyrus to improve cognitive functions in Alzheimer's disease patients. Over the course of 14 weeks, a significant improvement in cognitive performance was observed in both patients.
tACS as a promising therapeutic option for improving cognitive function in mild cognitive impairment: A direct comparison between tACS and tDCS (2021) Jiheon Kim, Hansol Kim, Hyewon Jeong, Daeyoung Roh, Do Hoon Kim Hallym University, Chuncheon, Republic of Korea	This sham-controlled, double-blind study reveals the superiority of 40 Hz tACS over tDCS in improving higher-order cognitive functions in patients with Mild Cognitive Impairment. The beneficial effect of tACS became more evident with increasing difficulty of the cognitive tests, demonstrating the positive effects of 40 Hz tACS on higher cognitive functions such as cognitive control, working memory, and decision-making.
Effects of 40 Hz transcranial alternating current stimulation (tACS) on cognitive functions of patients with Alzheimer's disease: a randomised, double-blind, sham-controlled clinical trial (2021) Dongsheng Zhou, Ang Li, Xingxing Li, Wenhao Zhuang, Yiyao Liang, Cheng-Ying Zheng, Hong Zheng, Ti-Fei Yuan Nantong University, Nantong, People's Republic of China	In this study, the authors have shown that bilateral temporal lobe stimulation over the course of 6 weeks significantly reduces plasma Amyloid-42 levels compared to a sham control group.

MIAMIND

frontiers | Frontiers in Human Neuroscience

TYPE Clinical Trial
PUBLISHED 02 June 2023
DOI 10.3389/fnhum.2023.1168673

OPEN ACCESS

EDITED BY
Marco Filardi,
University of Bari Aldo Moro, Italy

REVIEWED BY
Alberto Benussi,
University of Brescia, Italy
Jason R. Taylor,
The University of Manchester, United Kingdom

*CORRESPONDENCE
Davide Cappon
✉ davidebaloscappon@hsl.harvard.edu

RECEIVED 17 February 2023
ACCEPTED 27 April 2023
PUBLISHED 02 June 2023

CITATION
Cappon D, Fox R, den Boer T, Yu W, LaGanke N, Cattaneo G, Perellón-Alfonso R, Bartrés-Faz D, Manor B and Pascual-Leone A (2023) Tele-supervised home-based transcranial alternating current stimulation (tACS) for Alzheimer's disease: a pilot study. *Front. Hum. Neurosci.* 17:1168673. doi: 10.3389/fnhum.2023.1168673

Tele-supervised home-based transcranial alternating current stimulation (tACS) for Alzheimer's disease: a pilot study

Davide Cappon[1,2,3]*, Rachel Fox[1,2], Tim den Boer[1], Wanting Yu[1], Nicole LaGanke[1], Gabriele Cattaneo[4,5,6], Ruben Perellón-Alfonso[7,8], David Bartrés-Faz[7,8], Brad Manor[1,9] and Alvaro Pascual-Leone[1,2,3]

[1]Hinda and Arthur Marcus Institute for Aging Research at Hebrew SeniorLife, Boston, MA, United States, [2]Deanna and Sidney Wolk Center for Memory Health at Hebrew SeniorLife, Boston, MA, United States, [3]Department of Neurology, Harvard Medical School, Boston, MA, United States, [4]Institut Guttmann, Institut Universitari de Neurorehabilitació Adscrit a la UAB, Barcelona, Spain, [5]Departament de Medicina, Universitat Autònoma de Barcelona, Bellaterra, Spain, [6]Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Badalona, Spain, [7]Department of Medicine, Faculty of Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona, Barcelona, Spain, [8]Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), Barcelona, Spain, [9]Department of Medicine, Harvard Medical School, Boston, MA, United States

Background: Over 55 million people worldwide are currently diagnosed with Alzheimer's disease (AD) and live with debilitating episodic memory deficits. Current pharmacological treatments have limited efficacy. Recently, transcranial alternating current stimulation (tACS) has shown memory improvement in AD by normalizing high-frequency neuronal activity. Here we investigate the feasibility, safety, and preliminary effects on episodic memory of an innovative protocol where tACS is administered within the homes of older adults with AD with the help of a study companion (HB-tACS).

Methods: Eight participants diagnosed with AD underwent multiple consecutive sessions of high-definition HB-tACS (40 Hz, 20-min) targeting the left angular gyrus (AG), a key node of the memory network. The Acute Phase comprised 14-weeks of HB-tACS with at least five sessions per week. Three participants underwent resting state electroencephalography (EEG) before and after the 14-week Acute Phase. Subsequently, participants completed a 2–3-month Hiatus Phase not receiving HB-tACS. Finally, in the Taper phase, participants received 2–3 sessions per week over 3-months. Primary outcomes were safety, as determined by the reporting of side effects and adverse events, and feasibility, as determined by adherence and compliance with the study protocol. Primary clinical outcomes were memory and global cognition, measured with the Memory Index Score (MIS) and Montreal Cognitive Assessment (MoCA), respectively. Secondary outcome was EEG theta/gamma ratio. Results reported as mean ± SD.

Results: All participants completed the study, with an average of 97 HB-tACS sessions completed by each participant; reporting mild side effects during 25% of sessions, moderate during 5%, and severe during 1%. Acute Phase adherence was 98 ± 6.8% and Taper phase was 125 ± 22.3% (rates over 100% indicates participants completed more than the minimum of 2/week). After the Acute Phase, all participants showed memory improvement, MIS of

10.3389/fnhum.2023.1168673

7.25 ± 3.77, sustained during Hiatus 7.00 ± 4.90 and Taper 4.63 ± 2.39 Phases compared to baseline. For the three participants that underwent EEG, a decreased theta/gamma ratio in AG was observed. Conversely, participants did not show improvement in the MoCA, 1.13 ± 3.80 after the Acute Phase, and there was a modest decrease during the Hiatus −0.64 ± 3.28 and Taper −2.56 ± 5.03 Phases.

Conclusion: This pilot study shows that the home-based, remotely-supervised, study companion administered, multi-channel tACS protocol for older adults with AD was feasible and safe. Further, targeting the left AG, memory in this sample was improved. These are preliminary results that warrant larger more definite trials to further elucidate tolerability and efficacy of the HB-tACS intervention. NCT04783350.

Clinical trial registration: https://clinicaltrials.gov/ct2/show/NCT04783350?term=NCT04783350&draw=2&rank=1, identifier NCT04783350.

KEYWORDS

Alzheimer's disease, neuromodulation, transcranial alternating current stimulation (tACS), memory, cognition, electroencephalography (EEG)

Introduction

Over 55 million people worldwide are currently diagnosed with Alzheimer's disease (AD) and this number is predicted to nearly double by the year 2050, due to the aging population (Alzheimer's Association, 2022; World Health Organization, 2022). Early on in the course of their condition, AD patients typically have episodic memory loss, which is devastating to them as well as to their family and caregivers and is a useful indicator of how the disease will progress (El Haj et al., 2017; Abraham et al., 2020).

The targets of current pharmacological therapy for memory enhancement are beta-amyloid plaque (β-amyloid) deposition (Avgerinos et al., 2021; van Dyck et al., 2022), one of the pathophysiological hallmarks of AD, and the related dysregulation of the cholinergic system (Hampel et al., 2018). Alongside cholinergic dysregulation and β-amyloid accumulation, recent studies have shown that high frequency brain network activities that support successful memory encoding and recall of new information are altered decades before AD clinical onset, and the disrupted networks predict future pathology and brain atrophy (Koenig et al., 2005; Sperling et al., 2009; Babiloni et al., 2016; Palop and Mucke, 2016; Palop, 2020). Specifically, recent research has focused primarily on the role of gamma desynchronization (30–80 Hz) in AD manifestation, raising the possibility of exploiting it as a novel therapeutic target.

In animal models of AD, entrainment-induced restoration of gamma oscillations reduces the pathogenic load of β-amyloid and significantly improves behavior (Iaccarino et al., 2016; Adaikkan et al., 2019; Etter et al., 2019; Martorell et al., 2019). Further, neuroimaging evidence indicate that the AG is a key node in the memory network, and gray matter volume reduction in the AG has been linked to AD memory symptoms (Oh et al., 2013; Humphreys et al., 2021; van de Mortel et al., 2021). For the translation of these findings to humans, transcranial alternating current stimulation (tACS) has drawn interest for its ability to modulate cortical excitability and improve cognitive functions by safely modulating brain activity at a precise frequency in targeted brain structures (Zaehle et al., 2010; Antal and Paulus, 2013; Herrmann et al., 2013; Cappon et al., 2015, 2016). Recent initial studies in AD have demonstrated that the application of tACS at gamma frequency targeting key nodes of the memory network improved episodic memory and restored cholinergic neurotransmission (Benussi et al., 2021; Kim et al., 2021; Benussi et al., 2022; Zhou et al., 2022).

These recent findings are very encouraging for the application of tACS as a safe and effective intervention in patients with AD. However, studies to date have been limited by the fact that only a single tACS session was administered in a clinic-lab setting, which raises questions about how long memory effects will last and whether it will be possible to scale up tACS interventions for patients who cannot afford to travel to a specialized clinic center. Thus, there is an urgent need for a safe, effective, and more accessible home-based intervention for memory in AD.

We have previously developed an innovative methodology and demonstrated the safety and feasibility of providing a home-based, remotely supervised, study companion (e.g., caregiver, family member, friend) administered, multi-channel neuromodulation protocol for older adults (Cappon et al., 2022). This approach offers the opportunity to integrate therapeutic benefit into the daily life of participants and increasing access to tACS treatment. Caregivers are also empowered as they play a vital role as study companions directly assisting in treatment for their loved one. In the present pilot study, we report the results of a case series investigation including eight AD-diagnosed participants who received multiple sessions of home-based, remotely supervised, study companion administered, multi-channel tACS targeted at the left angular gyrus (L-AG). Assessing the intervention's safety, feasibility, and early clinical impact on episodic memory were the study's primary objectives.

10.3389/fnhum.2023.1168673

Materials and methods

Participants

Ten participants were enrolled in the study, with two participants (P004 and P009) withdrawing due to personal reasons unrelated to their participation in the trial. Eight participants diagnosed with AD received home-based transcranial alternating current stimulation (HB-tACS) from March 3rd, 2021 to June 3rd, 2022. Demographic and clinical characteristics of participants are presented in Table 1. All participants had amnestic impairment, verified by the study neurologist. Inclusion criteria for participants were: AD diagnosis (characterized by AD biomarker positivity and by in-person assessment by qualified health care personnel), over the age of 50, able to give consent, ability to read, write, and communicate in English, and able to identify an eligible study companion to participate with them in the study to administer the HB-tACS. Exclusion criteria included major psychiatric co-morbidities, dermatological conditions on the scalp, an inability to provide informed consent, or any contraindications to tACS (such as recent seizures, implanted medical devices, use of neuroactive drugs, etc.). Inclusion criteria for study companions were: at least 21 years old, self-reported computer proficiency, able to read, write, and communicate in English, willingness to learn how to administer HB-tACS, availability during weekdays of the study period to administer HB-tACS to the study participant, and a MoCA score of 27–30 to demonstrate their cognitive capacity. Study companions demographic characteristics and the number of HB-tACS sessions completed during the study are displayed in Table 2.

Study design

The study intervention consisted of multiple consecutive sessions of HB-tACS (40 Hz, 20-min) targeting the left angular gyrus (L-AG), a key node of the memory network. The protocol consisted of three phases: Acute Phase, Hiatus Phase, and the Taper Phase (see Figure 1). During the Acute Phase, the study companions conducted daily 20-min HB-tACS sessions for 14 weeks, with a minimum of five sessions each week (maximum of one session per day, seven sessions per week). A subset of three participants completed an optional resting state high-density electroencephalography (HD-EEG) visit at baseline and at the end of the acute phase. HD-EEG was included to gain insight into the underlying neurophysiological alterations induced by tACS, we opted to make it optional to minimize the burden of additional assessments since it wasn't a primary outcome of the present study. After the Acute Phase, participants and their study companions completed 3 months without any stimulation during the Hiatus Phase. The goal of the Hiatus Phase was to capture the extent to which any effects of HB-tACS on memory may have been sustained after 3 months without stimulation. Subsequently, in the Taper phase, we asked participants to complete 2–3 HB-tACS sessions per week over 3 months. The objective of this phase was to facilitate maintenance of any benefit derived from HB-tACS in the Acute Phase. Assessments were conducted at baseline and

TABLE 1 Participant demographics and clinical characteristics.

Participant	Age	Sex	Education (years)	Diagnosis	MoCA score (Baseline)	Memory Index Score (Baseline)
P001	79	M	19	Late onset AD	21	0
P002	79	M	19	Late onset AD	23	0
P003	88	F	16	AD	28	0
P006	79	F	16	AD	17	1
P007	71	M	18	AD	18	0
P008	66	M	16	Moderate AD	10*	1
P010	53	M	16	Early onset moderate AD	10*	0
P011	76	M	16	AD	22	4

*Please note P008 and P010 were more impaired in their baseline MoCA score, but as this is a pilot study, we sought to be more inclusive of our participants and their level of decline.

TABLE 2 Study companions demographic, level of education, and the number of sessions completed.

Study companion for	Age	Sex	Education (years)	Number of HB-tACS sessions completed	
				Acute Phase	Taper Phase
P001	78	F	16	70	48
P002	73	F	16	69	35
P003	88	M	18	64	20
P006	80	M	16	69	22
P007	64	F	16	79	24
P008	59	F	16	64	24
P010	53	F	16	68	26
P011	74	F	16	66	32

10.3389/fnhum.2023.1168673

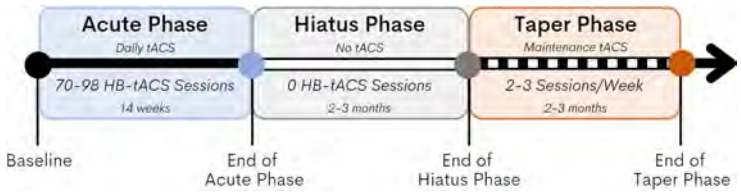

FIGURE 1
Study design. The Acute Phase lasted 14 weeks, the study companions conducted daily 20-min HB-tACS sessions with a minimum of five sessions per week (maximum of one session per day, seven sessions per week). The Hiatus lasted 3 months no HB-tACS sessions were administered. In the Taper Phase, we asked participants to complete 2–3 HB-tACS sessions per week over 3 months.

at the end of each of the three phases. Further, we monitored adherence to the HB-tACS protocol through all phases and a member of the research staff called the study companions once a week during the Acute and the Taper Phase to check on the participants' condition and to record any subjective effects of tACS on their memory.

Study procedures

The study was approved and monitored by an independent Institutional Review Board (Advarra, Inc.) and registered as clinical trial at clinicaltrials.gov (NCT04783350). All subject interaction was conducted either in-person at the Hebrew Rehabilitation Center in Boston, MA, or remotely via videoconferencing media. Prior to enrolling in the study, participants and study companions attended a 45-min phone screening to ensure they fulfilled eligibility criteria. If eligible, participants then completed an in-person screening visit comprised of a medication history, neurological exam, Clinical Dementia Rating (CDR), and a Montreal Cognitive Assessment (MoCA) (from which the Memory Index Score (MIS) is calculated) to assess global cognitive status. Participants were given the choice to undergo resting state HD-EEG recording if they were eligible for the study.

Optimized tACS current flow modeling to target the left angular gyrus

The specific tACS system used in this study is the Starstim®-Home Kit (Neuroelectrics Corp.). The Starstim® device includes a headcap that resembles a swimming cap with holes located where small electrodes can be attached and secured in place in the correct position on the scalp. These electrode holes are color- and number-coded so that electrode leads with corresponding colors coming from the tACS device are appropriately attached to the corresponding electrodes, eliminating the potential for accidental mismatching of the electrodes and the leads. Each session involved a multichannel tACS montage with maximal anodal current targeting the left AG administered via 6 NG Pistim electrodes (circular electrodes with a contact of area of 3.14 cm^2) using the Starstim® -Home system. Created using the Stimweaver® algorithm (Ruffini et al., 2014), the montage was specifically designed to optimize stimulation over the left AG and at the same time minimize off target stimulation effects based on a standard brain model (see **Figure 2**). During each sessions the currents delivered by any single electrode did not exceed 2.0 mA, well below recommended safety limits (Antal et al., 2017). The average E-field normal component on the target was En = 0.24 V/m, and En = 0.10 V/m on the surrounding regions. For all participants, current intensity was ramped up over 60 s, then sustained at the

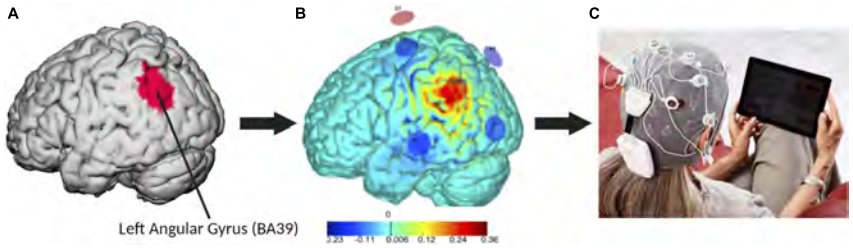

FIGURE 2
The multichannel tACS intervention for AD. **(A)** The red area on the default brain model represents the left angular gyrus, or Brodmann's area 39 (BA39). This target area was used to develop the current flow modeling and electrode montage. **(B)** The optimized six-electrode montage developed in this study to target the left AG (anode shown in red, cathodes in blue) and normal component of the electric field to the cortex induced by the montage (V/m). **(C)** Set-up of the Neuroelectrics Starstim© Home Kit headcap and tablet.

stimulation intensity for 20 min, then ramped down over 60 s. This standard approach is both well-tolerated and safe in older adults (Cappon et al., 2022).

Home-based study companion-led tACS administration

After being enrolled, participants and their study companions came to the laboratory on three consecutive days to receive hands-on instruction in the administration of tACS. On the first day, study companions were given an overview of the Starstim®-Home Kit (Neuroelectrics Corp.) tACS equipment by a trained research staff member. On the same day, research staff administered the first tACS session to the study participant, instructing the study companion who observed the administration. On the second day, the study companion was encouraged to carry out as much of the tACS session as possible without guidance from the research staff. However, the study companion was allowed to ask for assistance as necessary, and research staff corrected them if they made any mistakes. On the third day, study companions independently administered the stimulation to the participant, while research staff assessed the performance of the study companion using an evaluation check list adapted from Charvet et al. (2015). Participants then brought the HB-tACS device home to complete stimulation sessions independently with remote supervision from research staff. For further explanation of this HB-tES training and supervision program, please see Cappon et al. (2022) and den Boer et al. (2022).

Study outcomes

Safety

Side effects questionnaire and adverse events

Before and after each home-based tACS session, participants were asked to report any side effects on questionnaires that were implemented using the Starstim®-Home system smart tablet. Based on previous published safety guidelines, participants were asked to report on any physical sensations, scalp abnormalities, cognitive changes, or other symptoms that were present (Antal et al., 2017). A list of the side effects that were asked about can be found in **Table 3**. Participants were asked to report on the intensity of the side effects, by stating whether their experience for each of the side effects was: absent, mild, moderate, or severe. These responses were received via email by research staff through a study email inbox in real-time. If participants indicated a moderate or severe experience of a side effect prior to the tACS, access to the stimulation was blocked until study staff could ensure that the participant was fit for stimulation.

Feasibility

Adherence and compliance

The adherence to the treatment schedule, measured as the ratio of completed HB-tACS sessions to the required sessions as prescribed in the study schedule (a minimum of five sessions per week in the Acute Phase, and a minimum of two sessions per week in the Taper Phase), was used to evaluate the feasibility of the home-based tDCS protocol. This allowed us to capture how HB-tACS session administration can fit into the daily life of the participant and study companion and help determine if this method is feasible for future use in an older adult population.

Clinical outcomes

Clinical outcomes focused on memory and global cognitive functioning, as measured by the Memory Index Score (MIS) and the full Montreal Cognitive Assessment (MoCA), respectively. Research staff who have completed MoCA training and obtained certification administered these examinations to ensure consistency in the administration. They were assessed at baseline, after the Acute Phase, after the Hiatus Phase, and after the Taper Phase. At the end of each of the three phases, data were acquired and stored using a REDCap database, a secure platform for storing data and generating reports.

Montreal cognitive assessment (MoCA)

The MoCA is a clinician-rated screening test consisting of 12 items across different cognitive domains, including visuospatial/executive functioning, animal naming, memory, attention, language, abstraction, delayed recall, and orientation. It can be administered within 10 min, resulting in a maximum total score of 30 points. Lower scores indicate greater cognitive decline, with MoCA scores below 26 indicating the presence of mild cognitive decline (Nasreddine et al., 2005). The 5-min telehealth MoCA was used over the telephone or videoconferencing media as a way to assess cognition without requiring the participant to travel to the research laboratory (Wong et al., 2015). The MoCA 5-min is comprised of five sub-tests that were taken from the MoCA and look at different cognitive domains: orientation, executive functions/language, verbal learning and memory, and attention. This shortened 5-min MoCA is highly correlated in score with the full 12-item MoCA (Wong et al., 2015). The version of the MoCA (including MIS words) was randomized for each assessment to minimize practice effects, though we acknowledge that there is still a potential impact of practice effects (Lei et al., 2022).

Memory Index Score (MIS)

The MIS is a measure of delayed recall memory that is calculated from the MoCA (either the full or 5-min). Five unrelated words are spoken to the participant twice, and each time they are repeated by the participant to facilitate the encoding process. After a 5-min delay, participants are asked to recall the five words. If freely recalled without a cue, 3 points per word is earned toward the MIS. If recalled with a categorical cue, 2 points per word is earned. If recalled when presented with multiple choice options, 1 point per word is earned, for a maximum possible score of 15 points (Julayanont et al., 2014). Thus, the MIS score computation includes points for both the free recall and cued recall conditions, unlike the MoCA, which only accounts for points for the delayed free recall condition. Due to how the MIS is calculated, a large effect can be seen in a participant's MIS score, but only minimal changes seen in the MoCA overall score.

EEG recording and analysis

For those participants who opted in to this part of the study, 5 min of eyes closed resting state high density EEG were

10.3389/fnhum.2023.1168673

recorded continuously using a 256 channels HydroCel Geodesic Sensor Net (Electrical Geodesic Inc., Eugene, OR, USA) at 1 KHz sampling rate and bandpass filtered 0.3 to 500 Hz. Electrode impedance was kept under 50 Kohm. EEG data was pre-processed using EEGLAB (Delorme and Makeig, 2004) and custom-made Matlab (The MathWorks, Inc., Natick, MA, USA) scripts. First, face and neck electrodes were removed from the data. Then we retained only 62 of the remaining scalp electrodes that could be directly mapped to the 10–10 international electrode position system (Luu and Ferree, 2005). Line noise (i.e., 60 Hz and harmonics) was then attenuated using the Zapline algorithm (de Cheveigné, 2020). Excessively noisy or disconnected electrode channels were spline interpolated and data was re-referenced to the average of all channels. Next, the continuous data was segmented into non-overlapping two-second-long epochs. All epochs were visually inspected and those that contained any artifacts were removed. The cleaned preprocessed data was then used for source reconstruction in Brainstorm (Tadel et al., 2019). For each subject a forward model was estimated via the openMEEG algorithm (Kybic et al., 2005) using the default settings (i.e., 3 layers with 1,922 vertices each; skull and scalp conductivities of 1 and brain conductivity of 0.0125; adaptative integration), and based on the MNI ICBM152 average brain template (Mazziotta et al., 1995). The inverse solution was estimated using the minimum norm imaging method (Salmelin and Baillet, 2009). Sources were then computed as current density maps for constrained orientations only (i.e., normal to cortex). We aimed to determine how much the HB-tACS intervention impacted the oscillatory power of gamma oscillations in the left angular gyrus. In source space, we then defined a single region of interest (ROI) containing the left angular gyrus, based on the Desikan-Kiliani cortical parcellation (Desikan et al., 2006). Power spectral density estimates were then extracted from all voxels of this ROI using the Welch method (Welch, 1967) and averaged. Finally, we computed relative power frequency bands for theta (4–7.9 Hz), alpha (8–13.9 Hz), beta (14–29.9 Hz), and gamma (30–50 Hz), and the ratio of theta to gamma.

Results

Safety

Side effects questionnaire and adverse events

All side effects reported from baseline to the end of the Taper Phase were transient. The most frequently reported side effects were mild and reported during less than 1% of sessions, as shown in **Table 3**. The two exceptions to this, however, were the side effects "Sensations under the electrode" and "Sleepiness." Participants reported sensations under the electrode (such as a tingling or an itching feeling) during 25.6% of sessions. Feeling sleepy as a consequence of the stimulation was reported during 4% of sessions, and found to be transient (**Table 4**). Overall, side effects were reported in 33.4% of sessions, with participants reporting mild side effects during 26.6% of sessions, moderate side effects during 5.6% of sessions, and severe side effects during 1.2% of sessions as seen in **Table 3**. Upon the participants reporting a moderate or severe side effect, research staff was notified and would immediately contact participants and gather more information about the side effect and how they were currently feeling. After this contact, research staff would report the situation to the medically responsible study physician, who would assess the event and advise participants on how to proceed. The monitoring of adverse events showed that no adverse events occurred during the course of the study.

Feasibility

Adherence

All eight participants and their study companions had very high adherence to our protocol, collectively completing 780 HB-tACS sessions between baseline and the end of the Taper Phase. The adherence of individual participants is shown in **Table 5**. The average percent adherence to the tACS protocol for the Acute Phase was 98% ± 6.8%, showing that participants and their study companions were able to strongly comply with daily tACS. The average adherence in the Taper Phase is encouraging, as when participants were empowered to choose the number of tACS sessions they completed, nearly all completed more than the

TABLE 3 Total incidence of side effects and their severity inquired about in the side effect questionnaires pre and post tACS.

Side effect	Mild		Moderate		Severe		Percentage of sessions
	Pre	Post	Pre	Post	Pre	Post	
Headache	0	0	0	0	0	0	0%
Neck pain	0	2	0	0	0	0	0.2%
Scalp pain	0	2	1	2	0	1	0.81%
Scalp burns	3	1	0	2	0	1	0.9%
Sensations under the electrode	3	162*	1	27	0	6	25.6%
Skin redness	1	3	0	0	0	0	0.5%
Sleepiness	1	18	0	11	0	1	4%
Trouble concentrating	0	9	0	0	0	0	1.2%
Acute mood change	1	1	0	0	0	0	0.2%
Other	0	0	0	0	0	0	0%
Total %	1.2%	25.4%	0.2%	5.4%	0%	1.2%	33.4%

*P007 and P010 reported the highest frequency of "sensations under the electrode", accounting for over 79% of reports (see **Table 4**).

10.3389/fnhum.2023.1168673

minimum of 2 sessions per week, with an average adherence of 125% ± 22.3%, or completing an average of 2.5 sessions each week. Adherence rates over 100% indicates participants completed more than the minimum required sessions per week.

Cognitive outcomes

Memory Index Score (MIS)

All participants showed memory improvement at the end of the Acute Phase, with an average improvement of 7.25 ± 3.77 points. This was sustained during the Hiatus 7.00 ± 4.90 and Taper 4.63 ± 2.39 phases compared to baseline. Even at the end of the study period, all participants had maintained some of the original benefit from the Acute Phase. These average changes in scores are showed in **Figure 3**, with individual scores in **Figure 4A**. The individual MIS scores at each assessment point are also displayed in **Table 6**.

Montreal Cognitive Assessment (MoCA)

Participants did not show any dramatic change in the MoCA at the end of the Acute Phase, with an average of 1.13 ± 3.80 points. There was a modest decrease during the Hiatus −0.64 ± 3.28 and Taper −2.56 ± 5.03 Phases from baseline. These average changes in scores are showed in **Figure 3**, with individual scores in **Figure 4B**. The individual MoCA scores at each assessment point are also displayed in **Table 7**.

TABLE 4 Frequency with which participants reported sensations under the electrodes.

Participant	# Reported sensations under the electrodes
P001	4
P002	1
P003	8
P006	46
P007	63
P008	1
P010	65
P011	11

TABLE 5 Participant adherence to tACS protocol in the Acute Phase and Taper Phase.

Participant	Acute Phase adherence % (minimum 5 sessions/week)	Taper Phase adherence % (minimum 2 sessions/week)
P001	100%	160%*
P002	99%	138%*
P003	91%	91%
P006	99%	100%
P007	113%*	123%*
P008	91%	133%*
P010	97%	139%*
P011	94%	118%*
Average	98% (6.8)	125% (22.3)

*Adherence over 100% indicates participants completed more than the minimum required sessions per week.

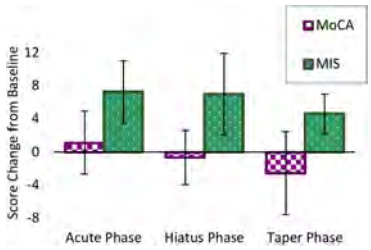

FIGURE 3
Participant average score change from baseline on Memory Index Score (MIS) (green) and MoCA (purple). While global cognition measured by the MoCA stayed stable with a modest decrease over time, episodic memory measured by the MIS showed great improvement from baseline after the Acute Phase, which was sustained even after the Taper Phase.

Neurophysiological outcome

EEG resting-state

As the EEG was optional in this study, only a subset of three participants chose to take part: P007, P008, and P011. Theta/gamma ratio, which was assessed in source space within the ROI including the left angular gyrus (L-AG), decreased in all 3 participants after the Acute Phase (**Figure 4C**).

Discussion

In the present study, we adopted our recently developed method of providing a home-based, remotely supervised, study companion (e.g., caregiver, family member, friend) administered, multi-channel neuromodulation protocol for older adults (Cappon et al., 2022).

We monitored side effects throughout the experiment using a customized side-effect questionnaire administered on a tablet. No serious adverse events occurred across the total of 780 HB-tACS sessions. The side effects recorded during the study were mostly mild and transient. Specifically, of the 31% of sessions in which side effects were reported, participants reported 80% mild, 17% moderate and 3% severe side effects on an intensity rating with a range of mild, moderate, and severe options. This is in line with previous research that showed that tACS was well-tolerated and that no serious adverse events were reported when tACS was administered in accordance with the safety guidelines (Antal et al., 2017).

We found that the study companions effectively conducted the tACS sessions at home after receiving instruction and training. This was demonstrated by the fact that, on average, 68.6 tACS sessions in the Acute Phase and 28.9 sessions in the Taper Phase were delivered successfully by study companions. This is promising for future research as it shows that the training in tACS administration can empower study companions to conduct the stimulation sessions themselves in the home of the study participants and that this approach can free up research staff members from having to conduct the tACS sessions, thereby

10.3389/fnhum.2023.1168673

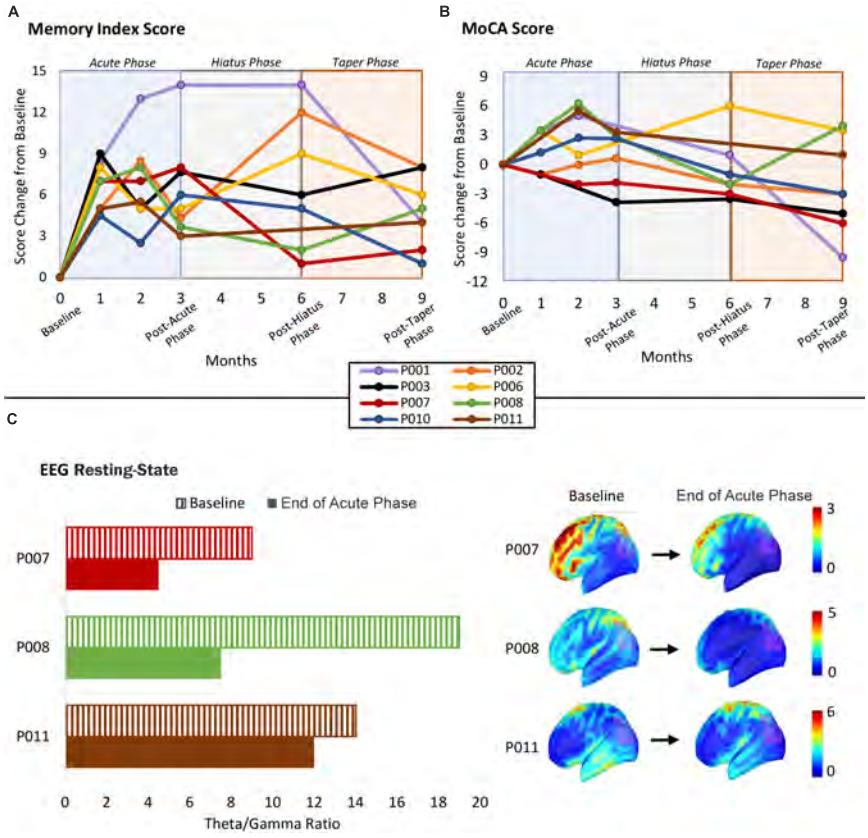

FIGURE 4
(A) Individual participant MIS scores over the course of the study duration. **(B)** Individual participant MoCA scores over the course of the study duration. Shortened 5-min MoCA were administered via videoconferencing media to assess potential short-term effects of the stimulation on global cognitive status and any incremental change at 1 and 2 months of the Acute Phase. **(C)** EEG results: theta/gamma ratio at baseline, and after the Acute Phase of tACS intervention course at the end of Acute Phase. Left panel shows a bar graph of each participant's theta/gamma ratio at Baseline and at the end of the Acute Phase within the defined left angular gyrus ROI (see in Methods; EEG recording and analysis section). Right panel shows the distribution of theta/gamma ratio in source space and projected onto the MNI template; the red transparent contour highlights the ROI from which the bar graph data was extracted.

substantially increasing the intervention's scalability and access. High adherence levels observed throughout the Acute Phase and Taper Phase provide evidence for the feasibility of the HB-tACS approach. The 125% adherence during the Taper Phase indicates that study companions were administering more sessions than the minimum amount requested by research staff. These findings are remarkable given that better treatment outcomes depend on higher levels of adherence and considering that individuals with chronic conditions frequently lose interest in following their prescribed treatment plan (Brown and Bussell, 2011).

All eight participants showed improvement in memory over the course of the trial. The improvement in memory at the end of the Acute Phase (7.38 points in the MIS score) was sustained 3 months later after the Hiatus Phase (7.00 points) and 6 months later after the Taper Phase (4.63 points). The episodic memory enhancement that was maintained at the end of the Taper Phase relative to baseline is clinically encouraging given the progressive nature of episodic memory deficits in AD. However, given the lack of control group in this study, it is impossible to determine whether these effects were due to the stimulation or due to some other element of trial participation. Importantly, potential practice effects on the MIS test were minimized using different memory stimuli during each iteration of the test. In fact, if memory from a previous run of the test would have influenced performance, it likely will have been in a negative fashion through proactive interference. However, it is possible that practice effects could have had a potential impact.

10.3389/fnhum.2023.1168673

TABLE 6 Memory Index Scores (MIS) for all participants and comparisons to baseline.

Participant	Baseline	Acute Phase month 1		Acute Phase month 2		Post-acute phase		Post-hiatus phase		Post-taper phase	
	Score	Score	Score change from baseline	Score	Score change from baseline	Score	Score change from baseline	Score	Score change from baseline	Score	Score change from baseline
P001	0	8.5	+ 8.5	13	+13	14	+ 14	14	+ 14	4	+4
P002	0	5	+ 5	8.5	+ 8.5	11	+ 11	12	+ 12	8	+8
P003	0	9	+ 9	5	+5	6	+ 6	6	+6	7	+ 7
P006	1	9	+ 8	6	+5	6	+ 5	10	+ 9	7	+6
P007	0	7	+ 7	7	+7	8	+ 8	1	+1	2	+ 2
P008	1	8	+ 7	9	+8	6	+ 5	3	+2	6	+ 5
P010	0	4.5	+ 4.5	2.5	+ 2.5	7	+7	5	+ 5	1	+1
P011	4	9	+ 5	9.5	+ 5.5	6	+2	N/A	N/A	8	+ 4

TABLE 7 MoCA scores for all participants during the course of the study and comparisons to baseline.

Participant	Baseline	Acute Phase month 1		Acute Phase month 2		Post-acute phase		Post-hiatus phase		Post-taper phase	
	Score	Score	Score change from baseline	Score	Score change from baseline	Score	Score change from baseline	Score	Score change from baseline	Score	Score change from baseline
P001	21	24.5	+ 3.5	26	+5	27	+ 6	22	+ 1	11.5	−9.5
P002	23	22	−1	23	+ 0	25	+ 2	21	−2	20	−3
P003	28	27	−1	23	−5	23	−5	24.5	−3.5	20.5	−7.5
P006	17	20.5	+ 3.5	18	+1	16	−1	23	+ 6	20.5	+ 3.5
P007	18	15	−3	16	−2	15	−3	15	−3	12	−6
P008	10	13.5	+ 3.5	16.5	+ 6.5	14	+ 4	8	−2	14	+ 4
P010	10	11.5	+ 1.5	12.5	+ 2.5	14	+ 4	9	−1	7	−3
P011	22	24	+ 2	27.5	+ 5.5	24	+ 2	N/A	N/A	23	+ 1

It is worth noting that the MoCA scores remained the same even when MIS scores increased. This is in line with previous literature that suggests that targeting domains of cognitive functioning with non-invasive brain stimulation may be a net zero-sum game (Brem et al., 2014). This is something that merits further investigation in larger, well controlled trials.

Finally, the observed EEG changes in the theta/gamma ratio may have a potential relation to the observed behavioral improvement and might indicate neurophysiological changes induced by repeated tACS exposure. Specifically, it has been shown that increased theta/gamma ratio is associated with memory impairment in AD (Moretti et al., 2009) and it is predictive of progression from MCI to AD (Moretti et al., 2011). Our results could potentially indicate a relation between a decrease in theta/gamma ratio and improvement in memory function. However, these are very preliminary results, and our small sample size for the EEG portion of this study makes it difficult to draw any robust conclusions. Further investigation is required to understand the relation between the behavioral and neurophysiological effects of HB-tACS targeted at the AG.

As a feasibility trial testing a novel memory-focused intervention for AD, our study has relevant limitations. First, the sample size is small. A partial explanation for this is that the novelty of this intervention approach required a preliminary pilot study. However, this small sample does limit the generalizability of the feasibility findings for this intervention. Secondly, as the first study of its kind, it was impractical to enroll a control group of AD patients who did not receive tACS or that received sham tACS. Therefore, it is impossible to account for potential placebo effects. Thirdly, the methodology for measuring changes in memory functioning was limited to a singular index. Future studies should aim to encompass a larger breadth of memory measurements to build a more well-rounded idea of how memory was impacted. Fourthly, the results of the EEG source localization are constrained by the elimination of a large number of electrodes due to noise, and the EEG was only assessed in a subset of participants, a too small sample to allow any conclusions to be drawn. Despite these limitations, this study provides relevant data for future tACS investigations in AD. Future studies should build off the present pilot study and aim to enroll higher numbers of patients and include appropriate controls to test clinical efficacy.

Conclusion

This pilot study shows the feasibility and preliminary efficacy of a novel, home-based, remotely supervised, study companion-led, multi-channel neuromodulation intervention for older adults with AD. We observed an improvement in memory scores throughout the duration of the intervention while global cognition was relatively stable, indicating that future interventions might benefit from multifocal neuromodulation targeting multiple cognitive domains. Future additional randomized controlled studies will be required to determine the efficacy of HB-tACS intervention. Our findings, which show safety and high adherence to this intervention are encouraging for continuing research with home-based neuromodulatory interventions for memory functioning in AD. Further, the preliminary evidence about memory improvements and potential decreases in the theta/gamma ratio merit larger and ultimately better controlled trials to more definitively determine the effect of tACS on memory functioning and on the underlying brain activity in AD. Finally, the differential effects on memory and global cognition should be further explored by, for example, administering complex neuromodulation interventions that target multiple cognitive domains simultaneously.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study involving human participants was approved and monitored by an independent Institutional Review Board (Advarra, Inc.). The patients/participants provided their written informed consent to participate in this study.

Author contributions

DC: conceptualization and design of the study, analysis and interpretation of data, writing of the original manuscript draft, reviewing and editing, and supervision. RF: acquisition of data, writing of the original draft, reviewing and editing, figures preparation, and editing. TB: acquisition of data, writing of the original manuscript draft, and reviewing and editing. WY and NL: acquisition of data, and writing—review and editing. GC: writing—review and editing. RP-A: analysis of data, interpretation of data, and writing—review and editing. DB-F: interpretation of data, and writing—review and editing. BM: conceptualization and design of the study, interpretation of data, and writing—review and editing. AP-L: conceptualization and design of the study, interpretation of data, writing of the original manuscript draft, reviewing and editing of manuscript, and supervision. All authors contributed to the article and approved the submitted version.

Funding

DC was partially supported by the Brain and Behavior Research Foundation (30772) and a grant from the NIH (R01 AG076708). AP-L and BM were partially supported by grants from the NIH (R01 AG059089 and R01 AG076708) and the Bright Focus Foundation. DB-F was supported by an ICREA Académia 2019 award. GC was partially supported by Fundació La Marató de TV3 (202129-30-31 and 202211-30-31). RP-A was supported

by a fellowship from "La Caixa" Foundation (ID 100010434; Fellowship code: 390 LCF/BQ/DI19/11730050).

Conflict of interest

AP-L is a co-founder of Linus Health and TI Solutions AG; serves on the scientific advisory boards for Starlab Neuroscience, Magstim Inc., Radiant Hearts, Tetraneuron, and MedRhythms; and is listed as an inventor on several issued and pending patents on the real-time integration of non-invasive brain stimulation with electroencephalography and magnetic resonance imaging.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abraham, M., Seidenberg, M., Kelly, D. A., Nielson, K. A., Woodard, J. L., Carson Smith, J., et al. (2020). Episodic memory and hippocampal volume predict 5-year mild cognitive impairment conversion in healthy apolipoprotein ε4 carriers. *J. Int. Neuropsychol. Soc.* 26, 733–738. doi: 10.1017/s1355617720000181

Adaikkan, C., Middleton, S. J., Marco, A., Pao, P.-C., Mathys, H., Kim, D. N.-W., et al. (2019). Gamma entrainment binds higher-order brain regions and offers neuroprotection. *Neuron* 102, 929–943.e8. doi: 10.1016/j.neuron.2019.04.011

Alzheimer's Association (2022). *2022 Alzheimer's disease facts and figures*. Chicago, IL: Alzheimer's Association.

Antal, A., Alekseichuk, I., Bikson, M., Brockmöller, J., Brunoni, A. R., Chen, R., et al. (2017). Low intensity transcranial electric stimulation: Safety, ethical, legal regulatory and application guidelines. *Clin. Neurophysiol.* 128, 1774–1809. doi: 10.1016/j.clinph.2017.06.001

Antal, A., and Paulus, W. (2013). Transcranial alternating current stimulation (tACS). *Front. Hum. Neurosci.* 7:317. doi: 10.3389/fnhum.2013.00317

Avgerinos, K. I., Ferrucci, L., and Kapogiannis, D. (2021). Effects of monoclonal antibodies against amyloid-β on clinical and biomarker outcomes and adverse event risks: A systematic review and meta-analysis of phase III RCTs in Alzheimer's disease. *Ageing Res. Rev.* 68:101339. doi: 10.1016/j.arr.2021.101339

Babiloni, C., Lizio, R., Marzano, N., Capotosto, P., Soricelli, A., Triggiani, A. I., et al. (2016). Brain neural synchronization and functional coupling in Alzheimer's disease as revealed by resting state EEG rhythms. *Int. J. Psychophysiol.* 103, 88–102. doi: 10.1016/j.ijpsycho.2015.02.008

Benussi, A., Cantoni, V., Cotelli, M. S., Cotelli, M., Brattini, C., Datta, A., et al. (2021). Exposure to gamma tACS in Alzheimer's disease: A randomized, double-blind, sham-controlled, crossover, pilot study. *Brain Stimul.* 14, 531–540. doi: 10.1016/j.brs.2021.03.007

Benussi, A., Cantoni, V., Grassi, M., Brechet, L., Michel, C. M., Datta, A., et al. (2022). Increasing brain gamma activity improves episodic memory and restores cholinergic dysfunction in Alzheimer's disease. *Ann. Neurol.* 92, 322–334. doi: 10.1002/ana.26411

Brem, A.-K., Fried, P. J., Horvath, J. C., Robertson, E. M., and Pascual-Leone, A. (2014). Is neuroenhancement by noninvasive brain stimulation a net zero-sum proposition? *NeuroImage* 85, 1058–1068. doi: 10.1016/j.neuroimage.2013.07.038

Brown, M. T., and Bussell, J. K. (2011). Medication adherence: WHO cares? *Mayo Clin. Proc.* 86, 304–314. doi: 10.4065/mcp.2010.0575

Cappon, D., den Boer, T., Jordan, C., Yu, W., Lo, A., LaGanke, N., et al. (2022). Safety and feasibility of tele-supervised home-based transcranial direct current stimulation for major depressive disorder. *Front. Aging Neurosci.* 13:765370. doi: 10.3389/fnagi.2021.765370

Cappon, D., D'Ostilio, K., Garraux, G., Bisiacchi, P., and Rothwell, J. (2015). Cortical modulation of automatic facilitation and inhibition by 10 Hz and 20 Hz transcranial alternating current stimulation (tACS). *Brain Stimulation* 8:356. doi: 10.1016/j.brs.2015.01.149

Cappon, D., D'Ostilio, K., Garraux, G., Rothwell, J., and Bisiacchi, P. (2016). Effects of 10 Hz and 20 Hz transcranial alternating current stimulation on automatic motor control.ă*Brain Stimulation* 9, 518–524. doi: 10.1016/j.brs.2016.01.001

Charvet, L., Shaw, M., Haider, L., Melville, P., and Krupp, L. (2015). Remotely-delivered cognitive remediation in multiple sclerosis (MS): Protocol and results from a pilot study. *Multiple Scler. J. Exp. Transl. Clin.* 1:205521731560962. doi: 10.1177/2055217315609629

de Cheveigné, A. (2020). ZapLine: A simple and effective method to remove power line artifacts. *NeuroImage* 207:116356. doi: 10.1016/j.neuroimage.2019.116356

Delorme, A., and Makeig, S. (2004). EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J. Neurosci. Methods* 134, 9–21. doi: 10.1016/j.jneumeth.2003.10.009

den Boer, T., Fox, R., Yu, W., LaGanke, N., Manor, B., Pascual-Leone, A., et al. (2022). "A remote training and supervision program from transcranial electrical stimulation in the home setting," in *Poster at the Cognitive Neuroscience Society Annual Meeting*, (San Francisco, CA). doi: 10.1177/1357633X19861830

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., et al. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 31, 968–980. doi: 10.1016/j.neuroimage.2006.01.021

El Haj, M., Roche, J., Gallouj, K., and Gandolphe, M.-C. (2017). Autobiographical memory compromise in Alzheimer's disease: A cognitive and clinical overview. *Gériatrie Psychol. Neuropsychiatr. Viellissement* 15, 443–451. doi: 10.1684/pnv.2017.0704

Etter, G., van der Veldt, S., Manseau, F., Zarrinkoub, I., Trillaud-Doppia, E., and Williams, S. (2019). Optogenetic gamma stimulation rescues memory impairments in an Alzheimer's disease mouse model. *Nat. Commun.* 10:5322. doi: 10.1038/s41467-019-13260-9

Hampel, H., Mesulam, M.-M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T., et al. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain* 141, 1917–1933. doi: 10.1093/brain/awy132

Herrmann, C. S., Rach, S., Neuling, T., and Strüber, D. (2013). Transcranial alternating current stimulation: A review of the underlying mechanisms and modulation of cognitive processes. *Front. Hum. Neurosci.* 7:279. doi: 10.3389/fnhum.2013.00279

Humphreys, G. F., Lambon Ralph, M. A., and Simons, J. S. (2021). A unifying account of angular gyrus contributions to episodic and semantic cognition. *Trends Neurosci.* 44, 452–463. doi: 10.1016/j.tins.2021.01.006

Iaccarino, H. F., Singer, A. C., Martorell, A. J., Rudenko, A., Gao, F., Gillingham, T. Z., et al. (2016). Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature* 540, 230–235. doi: 10.1038/nature20587

Julayanont, P., Brousseau, M., Chertkow, H., Phillips, N., and Nasreddine, Z. S. (2014). Montreal Cognitive Assessment Memory Index Score (MoCA-MIS) as a predictor of conversion from mild cognitive impairment to Alzheimer's disease. *J. Am. Geriatr. Soc.* 62, 679–684. doi: 10.1111/jgs.12742

Kim, J., Kim, H., Jeong, H., Roh, D., and Kim, D. H. (2021). tACS as a promising therapeutic option for improving cognitive function in mild cognitive impairment: A direct comparison between tACS and tDCS. *J. Psychiatr. Res.* 141, 248–256. doi: 10.1016/j.jpsychires.2021.07.012

Koenig, T., Prichep, L., Dierks, T., Hubl, D., Wahlund, L. O., John, E. R., et al. (2005). Decreased EEG synchronization in Alzheimer's disease and mild cognitive impairment. *Neurobiol. Aging* 26, 165–171. doi: 10.1016/j.neurobiolaging.2004.03.008

Kybic, J., Clerc, M., Abboud, T., Faugeras, O., Keriven, R., and Papadopoulo, T. (2005). A common formalism for the Integral formulations of the forward EEG problem. *IEEE Trans. Med. Imaging* 24, 12–28. doi: 10.1109/tmi.2004.837363

Lei, L., Lam, B., Lai, D., Bai, X., Li, J., Zou, Z., et al. (2022). Stability of montreal cognitive assessment in individuals with mild cognitive impairment: Potential influence of practice effect. *J. Alzheimers Dis.* 87, 1401–1412. doi: 10.3233/JAD-220003

Luu, P., and Ferree, T. (2005). Determination of the geodesic sensor nets' average electrode positions and their 10 – 10 international equivalents. Technical

Note. Available online at: https://www.researchgate.net/publication/266609828_Determination_of_the_Geodesic_Sensor_Nets%27_Average_Electrode_Positions_and_Their_10_-_10_International_Equivalents (accessed September 15, 2020).

Martorell, A. J., Paulson, A. L., Suk, H.-J., Abdurrob, F., Drummond, G. T., Guan, W., et al. (2019). Multi-sensory gamma stimulation ameliorates Alzheimer's-associated pathology and improves cognition. *Cell* 177, 256–271.e22. doi: 10.1016/j.cell.2019.02.014

Mazziotta, J. C., Toga, A. W., Evans, A., Fox, P., and Lancaster, J. (1995). A probabilistic atlas of the human brain: Theory and rationale for its development. *NeuroImage* 2, 89–101. doi: 10.1006/nimg.1995.1012

Moretti, D. V., Fracassi, C., Pievani, M., Geroldi, C., Binetti, G., Zanetti, O., et al. (2009). Increase of theta/gamma ratio is associated with memory impairment. *Clin. Neurophysiol.* 120, 295–303. doi: 10.1016/j.clinph.2008.11.012

Moretti, D. V., Frisoni, G. B., Fracassi, C., Pievani, M., Geroldi, C., Binetti, G., et al. (2011). MCI patients' EEGs show group differences between those who progress and those who do not progress to AD. *Neurobiol. Aging* 32, 563–571. doi: 10.1016/j.neurobiolaging.2009.04.003

Nasreddine, Z. S., Phillips, N. A., Bedirian, V., Charbonneau, S., Whitehead, V., Collin, I., et al. (2005). The montreal cognitive assessment, MoCA: A brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* 53, 695–699. doi: 10.1111/j.1532-5415.2005.53221.x

Oh, H., Madison, C., Villeneuve, S., Markley, C., and Jagust, W. J. (2013). Association of gray matter atrophy with age, β-amyloid, and cognition in aging. *Cereb. Cortex* 24, 1609–1618. doi: 10.1093/cercor/bht017

Palop, J. J. (2020). Network abnormalities and interneuron dysfunction in Alzheimer's disease. *Alzheimers Dement.* 16:e040396. doi: 10.1002/alz.040396

Palop, J. J., and Mucke, L. (2016). Network abnormalities and interneuron dysfunction in Alzheimer disease. *Nat. Rev. Neurosci.* 17, 777–792. doi: 10.1038/nrn.2016.141

Ruffini, G., Fox, M. D., Ripolles, O., Miranda, P. C., and Pascual-Leone, A. (2014). Optimization of multifocal transcranial current stimulation for weighted cortical pattern targeting from realistic modeling of electric fields. *NeuroImage* 89, 216–225. doi: 10.1016/j.neuroimage.2013.12.002

Salmelin, R., and Baillet, S. (2009). Electromagnetic brain imaging. *Hum. Brain Mapp.* 30, 1753–1757. doi: 10.1002/hbm.20795

Sperling, R. A., Laviolette, P. S., O'Keefe, K., O'Brien, J., Rentz, D. M., Pihlajamaki, M., et al. (2009). Amyloid deposition is associated with impaired default network function in older persons without dementia. *Neuron* 63, 178–188. doi: 10.1016/j.neuron.2009.07.003

Tadel, F., Bock, E., Niso, G., Mosher, J. C., Cousineau, M., Pantazis, D., et al. (2019). MEG/EEG group analysis with brainstorm. *Front. Neurosci.* 13:76. doi: 10.3389/fnins.2019.00076

van de Mortel, L. A., Thomas, R. M., and van Wingen, G. A. (2021). Grey matter loss at different stages of cognitive decline: A role for the thalamus in developing Alzheimer's disease. *J. Alzheimers Dis.* 83, 705–720. doi: 10.3233/jad-210173

van Dyck, C. H., Swanson, C. J., Aisen, P., Bateman, R. J., Chen, C., Gee, M., et al. (2022). Lecanemab in early Alzheimer's disease. *N. Eng. J. Med.* 388, 9–21. doi: 10.1056/nejmoa2212948

Welch, P. (1967). The use of fast Fourier transform for the estimation of power spectra: a method based on time averaging over short, modified periodograms. *IEEE Trans. Audio Electroacoust.* 15, 70–73. doi: 10.1109/TAU.1967.1161901

Wong, A., Nyenhuis, D., Black, S. E., Law, L. S. N., Lo, E. S. K., Kwan, P. W. L., et al. (2015). Montreal cognitive assessment 5-minute protocol is a brief, valid, reliable, and feasible cognitive screen for telephone administration. *Stroke* 46, 1059–1064. doi: 10.1161/strokeaha.114.007253

World Health Organization (2022). *Dementia*. Geneva: World Health Organization.

Zaehle, T., Rach, S., and Herrmann, C. S. (2010). Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PLoS One* 5:e13766. doi: 10.1371/journal.pone.0013766

Zhou, D., Li, A., Li, X., Zhuang, W., Liang, Y., Zheng, C.-Y., et al. (2022). Effects of 40 Hz transcranial alternating current stimulation (tACS) on cognitive functions of patients with Alzheimer's disease: A randomised, double-blind, sham-controlled clinical trial. *J. Neurol. Neurosurg. Psychiatry* 93, 568–570.

Published in final edited form as:
J Alzheimers Dis. 2022 ; 85(4): 1667–1676. doi:10.3233/JAD-215072.

Impact of 40 Hz Transcranial Alternating Current Stimulation on Cerebral Tau Burden in Patients with Alzheimer's Disease: A Case Series

Maeva Dhaynaut[a,1], **Giulia Sprugnoli**[b,1], **Davide Cappon**[b], **Joanna Macone**[b], **Justin S. Sanchez**[a,c], **Marc D. Normandin**[a], **Nicolas J. Guehl**[a], **Giacomo Koch**[d], **Rachel Paciorek**[b], **Ann Connor**[b], **Daniel Press**[b], **Keith Johnson**[a,c], **Alvaro Pascual-Leone**[e,f], **Georges El Fakhri**[a,1,*], **Emiliano Santarnecchi**[a,b,1,*]

[a]Gordon Center for Medical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

[b]Berenson-Allen Center for Noninvasive Brain Stimulation, Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

[c]Division of Nuclear Medicine and Molecular Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

[d]Santa Lucia Foundation, Rome, Italy

[e]Hinda and Arthur Marcus Institute for Aging Research and Deanna and Sidney Wolk Center for Memory Health, Hebrew SeniorLife, Boston, MA, USA

[f]Department of Neurology, Harvard Medical School, Boston, MA, USA

Abstract

Background: Alzheimer's disease (AD) is characterized by diffuse amyloid-β (Aβ) and phosphorylated Tau (p-Tau) aggregates as well as neuroinflammation. Exogenously-induced 40 Hz gamma oscillations have been showing to reduce Aβ and p-Tau deposition presumably *via* microglia activation in AD mouse models.

Objective: We aimed to translate preclinical data on gamma-induction in AD patients by means of transcranial alternating current stimulation (tACS).

*Correspondence to: Emiliano Santarnecchi, PhD, Gordon Center for Medical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA. Tel.: +1 617 667 0326, esantarnecchi@mgh.harvard.edu and Georges El Fakhri, PhD, DABR, Director, Gordon Center for Medical Imaging, Co-Director, Division of Nuclear Medicine & Molecular Imaging, Massachusetts General Hospital, Nathaniel & Diana Alpert Professor of Radiology, Harvard Medical School, Boston, MA, USA. Tel.: +1 617 953 5085, mwstpierre@mgh.harvard.edu.
[1]These authors contributed equally to this work.

APL and ES share a patent on the application of neuromodulation in Alzheimer's disease.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/21–5072r1).

SUPPLEMENTARY MATERIAL
The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-215072.

Methods: Four participants with mild-to-moderate AD received 1 h of daily 40 Hz (gamma) tACS for 4 weeks (Monday to Friday) targeting the bitemporal lobes (20 h treatment duration). Participant underwent Aβ, p-Tau, and microglia PET imaging with [^{11}C]-PiB, [^{18}F]-FTP, and [^{11}C]-PBR28 respectively, before and after the intervention along with electrophysiological assessment.

Results: No adverse events were reported, and an increase in gamma spectral power on EEG was observed after the treatment. [^{18}F]-FTPPET revealed a significant decrease over 2% of p-Tau burden in 3/4 patients following the tACS treatment, primarily involving the temporal lobe regions targeted by tACS and especially mesial regions (e.g., entorhinal cortex). The amount of intracerebral Aβ as measured by [^{11}C]-PiB was not significantly influenced by tACS, whereas 1/4 reported a significant decrease of microglia activation as measured by [^{11}C]-PBR28.

Conclusion: tACS seems to represent a safe and feasible option for gamma induction in AD patients, with preliminary evidence of a possible effect on protein clearance partially mimicking what is observed in animal models. Longer interventions and placebo control conditions are needed to fully evaluate the potential for tACS to slow disease progression.

Keywords

Amyloid; dementia; electroencephalography; gamma; neurostimulation; positron-emission tomography; protein clearance; protein misfolding; tau; transcranial electrical stimulation

INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia, accounting for 60–80% of all dementia cases, with its incidence and prevalence projected to increase [1]. Therapeutic options are very limited, with a few pharmacologic interventions that can temporarily slow down the memory loss and cognition impairment (e.g., cholinesterase inhibitors), but no available disease modifying treatments [2]. AD is characterized by diffuse extracellular amyloid-β (Aβ) plaques and phosphorylated Tau (p-Tau) intraneuronal aggregates. These aggregates interfere with neuron-to-neuron communication at the synaptic level, leading to neurodegeneration. Protein aggregates also cause the activation of microglia, the brain resident macrophage cells responsible of the neuroinflammation that, in turn, further promotes the neurodegeneration [3, 4]. Recent PET imaging studies suggest that progressive Aβ deposition can begin up to 20 years before the onset of clinical symptoms and stabilizes around the time that clinical symptoms become prominent [5]. p-Tau accumulates particularly in the mesial temporal lobes even in the absence of Aβ and spreads outside the temporal lobes following a different spatial trajectory than Aβ [6, 7]. Importantly, neurodegeneration and clinical symptoms are strongly correlated with the spread of p-Tau [6]. Consequently, evidence suggests that both Aβ and p-Tau play a critical role in AD pathogenesis even if their interdependency has not been fully disentangled yet [3, 8], making interventions that reliably and safely decrease intracerebral Aβ and p-Tau burden of marked clinical importance (Fig. 1).

Another consistent finding in patients with AD is a relative attenuation of fast oscillatory brain activity in the 30–80-Hz range, known as "gamma" activity [9]. Crucially, some of

the regions whose oscillatory activity in the gamma band, as well as fMRI connectivity, are altered in AD patients (i.e., regions of the default mode network, see Fig. 1), are the same regions affected by Aβ (i.e., lateral temporal lobe, angular gyrus, precuneus and medial frontal cortex), and partially by p-Tau (i.e., temporal pole, lateral temporal lobe, and angular gyrus), suggesting a link between oscillatory neural activity and protein accumulation [8, 10]. Recent preclinical work has demonstrated that exogenously-induced 40Hz gamma oscillations can reduce Aβ deposition *via* microglia activation and may also reduce p-Tau levels in a mouse model of AD (5XFAD) [11]. In presymptomatic AD mice, induction of gamma activity prevents subsequent neurodegeneration and behavioral impairments [12]. Those results suggest that gamma induction may represent a novel therapeutic approach for AD [13].Therefore, in the present case series study we aimed to test the safety and feasibility of translating the aforementioned preclinical findings on gamma induction to patients with AD, utilizing transcranial alternating current stimulation (tACS) as a tool to noninvasively entrain gamma oscillations, and PET imaging to assess protein clearance and neuroinflammation. tACS is a noninvasive brain stimulation technique able to entrain the underlying neuronal population by means of low-amplitude electrical current delivered at a specific frequency (e.g., 40Hz) over the scalp [14]. Neural entrainment is thought to promote oscillatory activity at the stimulation frequency and ultimately potentiate the cognitive or sensory functions supported by the targeted region and specific oscillatory activity [15, 16]. Interest on potential tACS effects on glial cells of the brain (i.e., microglia) have begun to arise recently, giving new hope for the treatment of neurodegenerative disorders [13].

In the present pilot trial, 40Hz tACS was administered 1h daily for 4 weeks (Monday to Friday) in a sample of mild-to-moderate AD patients, focusing the stimulation on the temporal lobes bilaterally. Longitudinal PET imaging was performed before and after the tACS treatment via C-Pittsburgh Compound B ([^{11}C]-PiB) to evaluate Aβ load, [^{18}F]-Flortaucipir ([^{18}F]-FTP) to quantify p-Tau pathology, and [^{11}C]PBR28 to assess microglia activation. The annual rate of change in global and regional Aβ and p-Tau ratio is rarely decreasing and is generally either nonsignificant or positive, typically on the order of 0.05 units/year, and asymptotes in patients with cognitive deficits and high Aβ and p-Tau levels [5, 17, 18]. Major spontaneous changes seem unlikely over the short time period between baseline and post-tACS treatment, allowing to capture potential beneficial effects of gamma-inducing tACS.

MATERIALS AND METHODS

Protocol

Five Aβ-positive patients with mild-to-moderate dementia due to AD were enrolled (Mini-Mental Score Examination (MMSE)=22.20, SD=4.5; male=3; mean age=70.2) and underwent 4 weeks (Monday to Friday, 20 sessions of 1h daily) of tACS at 40 Hz targeting the bilateral temporal lobes in hospital settings (Fig. 1). One participant did not complete the post stimulation evaluation due to scheduling issues, therefore was excluded from the analysis (final sample *N*=4; mean age=76 years, male=3; MMSE=20.7, SD=3.6). For more information on the sample including education, *APOE*, and BDNF status, see Table 1.

tACS uses low amplitude (usually delivered with a maximum of 2 mA per electrode) alternating sinusoidal currents *via* scalp electrodes to modulate cortical rhythmic activity in a frequency-specific manner [19, 20]. Bitemporal tACS was conceived as a way to induce focal stimulation over the temporal lobes given the typical Aβ and p-Tau protein distribution in these regions in the AD brain [6, 21], and on the basis of prior work by the PI using 40 Hz tACS in humans [15, 16, 22, 23]. Participant underwent baseline (pre-tACS) and follow-up (post-tACS) assessments composed of Aβ, p-Tau, and microglia PET imaging measured via [^{11}C]-PiB, [^{18}F]-FTP, and [^{11}C]-PBR28 on a Discovery MI (GE Healthcare) PET/CT scanner remaining below the limit of 50 mSv/year (36.9 mSv total study dose) as per the current safety guidelines on research exposure, along with cognitive testing and electroencephalography. [^{11}C]-PiB is an agent that binds specifically to Aβ [24], whereas [^{18}F]-FTP (previously known as T807 or AV-1451) binds with high affinity and selectivity to aggregated p-Tau pathology [25]. [^{11}C]-PBR28, a potent antagonist for mitochondrial translocator protein (TSPO), known to be upregulated in reactive microglia, demonstrates a good correlation between activated microglia and AD pathology [26].

At baseline and after the tACS treatment, patients underwent a cognitive assessment including measures of global cognition with specific focus on memory (Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) [27], baseline mean = 22.75, SD=8.2; MMSE [28], baseline mean = 20.7, SD = 3.6; Montreal Cognitive Assessment (MoCA) [29], baseline mean = 13.25, SD = 4.1), and measures of functional ability and independence (Activities of Daily Living (ADL) [30], baseline mean = 70, SD=2.6), to monitor safety of the proposed high-density tACS treatment regime also considering recent treatment-associated cognitive worsening reported for interventions targeting Aβ clearance pathways [31]. Additional details related to inclusion/exclusion criteria and cognitive assessment tools are reported as part of the Supplementary Material.

All participants gave written informed consent prior to participating in the study, registered on ClinicalTrials.gov (NCT03412604; PI Santarnecchi E). Patient's capacity to consent was established by a neurologist on the basis of the MMSE score and clinical evaluation during the consenting study visit. The research proposal and associated methodologies were approved by the local ethics committees (Beth Israel Deaconess Medical Center and Massachusetts Medical Center) in accordance with the principles of the Declaration of Helsinki.

tACS stimulation and EEG analysis

Stimulation was delivered at 40Hz for 1h using a Starstim 32-channels brain stimulation device (Neuroelectrics, Barcelona, Spain) in the morning for all participants, with each session starting between 8 to 12AM according to individual patients' schedules (Monday to Friday, 4 weeks, 20 tACS sessions in total). At the beginning of each session, the Starstim system was set up to stimulate both temporal lobes approximately corresponding to T8-T7 and P8-P7 in the 10/20 EEG system with a maximum intensity of 2mA for each electrode and 4mA total across all electrodes (Fig. 1), and impedance was checked. The daily tACS sessions started with resting-EEG recording (5' eyes open, 5' eyes closed), followed by 1h

of tACS at 40Hz. The stimulation intensity was ramped up over the first 60", kept constant through the entire session and ramped down over the last 60".

32-channel EEG data was recorded before and after each stimulation session using the Starstim device, allowing to monitor trajectory of change in gamma spectral power after each exposure to gamma-inducing tACS. Moreover, higher temporal and spatial resolution resting-state EEG was collected the week before and after the entire tACS treatment course (5' eyes open, 5' eyes closed) via a 64-channel actiCHamp EEG amplifier system (Brain Products GmbH), looking at changes in spectral power in three gamma sub-bands indexing low gamma (35–45Hz), mid gamma (45–60Hz), and gamma activity around the stimulation frequency (Stim γ =38–42 Hz), as well as slower oscillatory activity in the delta (1–4Hz), theta (4–8 Hz), alpha (9–13 Hz), and beta bands (14–30 Hz).

Neuroimaging data acquisition

All enrolled subjects underwent separate PET and MRI visits. Participants underwent three PET imaging acquisitions both before and after the tACS treatment (Aβ, p-Tau, and microglia PET). The [^{11}C]-PiB, [^{18}F]-FTP, and [^{11}C]-PBR28 tracers were synthesized and administered onsite at the Gordon Center for Medical Imaging at Massachusetts General Hospital (MGH) in Boston, MA, USA. A 3-dimensional structural T1-weighted BRAVO sequence was acquired for anatomical reference using a 3T General Electrics (GE) MRI (repetition time = 8240 ms; echo time = 3.24ms; inversion time = 450ms; flip angle = 12°; voxel size = 0.94 × 0.94 × 1mm^3 and matrix size = 256 × 256). MRI images were processed via FreeSurfer (FSv6.0) (http://surfer.nmr.mgh.harvard.edu) and manually edited to improve cortical surfaces and define standard regions of interest (ROI) for PET analysis [32]. PET data were acquired on a Discovery MI (GE Healthcare) PET/CT scanner in static mode at 75–105 min post bolus injection of 10 mCi (370 MBq) for [^{18}F]-FTP [33], 50–70 min post bolus injection of 15 mCi (555 MBq) in the case of [^{11}C]-PiB [34], and 60–90 min post bolus injection of 15 mCi (555 MBq) for [^{11}C]-PBR28 [35].

A low dose X-ray CT scan was performed right before each PET segment for attenuation correction and was obtained with the GE MI Discovery PET/CT scanner. All PET/CT scans were completed within a 2-week period both preceding and following the tACS treatment.

Image processing

All PET processing was performed with an inhouse developed Matlab software based on code and function from SPM8 (https://www.fil.ion.ucl.ac.uk/spm/software/spm8/) and FSL (https://fsl.fmrib.ox.ac.uk/fsl). PET images were motion corrected by realigning each frame to the first frame, co-registered to the corresponding baseline T1 image and FreeSurfer-derived ROIs were sampled.

PiB, FTP, and PBR28 retentions were expressed by standardized uptake values (SUV) normalized with a specific reference regions for each tracer (SUVr) providing more stable estimates for longitudinal studies [36]. Reference region-based analyses of [^{11}C]-PiB uptake were performed using the cerebellum cortex as a reference tissue [34], whereas for [^{18}F]-FTP the white matter was used instead [37, 33] and for [^{11}C]-PBR28 the uptake of the occipital cortex was employed. SUVr maps generated for these three tracers are shown in

Fig. 2. Subjects were defined as Aβ positive if the global Aβ burden in a large neocortical aggregate (FS-derived "FLR" regions, composed by frontal, lateral parietal and temporal, and retrospenial regions) using PiB SUVr was equal to or greater than 1.25 [25, 38].

PET statistical analysis

The results are expressed as delta SUVr: percent change in SUVr from baseline to post-tACS follow-up. Test-retest reliability of PET measurement has been demonstrated to be high, with a variability < 2% in the temporal lobes (Standard deviation, SD, at 4-week test-retest measurement = 1.97%) for [^{18}F]-FTP SUVr [39], and 3 to 5% for [^{11}C]-PiB SUVr [40]. As for [^{11}C]-PBR28, the cut-off of 2.47% determined by Nair et al. [41] when using the whole brain as reference region was used, as no test-retest study has been performed using the occipital cortex. Longitudinal comparison of individual SUVr data were interpreted as significant when showing a change (increase/decrease) higher than the aforementioned normative estimates of test-retest error.

RESULTS

Participants completed the study and tolerated the intervention without reporting any adverse events and attended 95% of the study visits (75/80 daily tACS visits, 5 sessions missed in total distributed across 3 patients), showing excellent treatment compliance even though the study required a high number of study visits (26 visits considering intervention and pre and post assessment visits) to be completed at the hospital (BIDMC, MGH). No seizures/signs of epileptiform activity were detected at the clinical assessment nor during the EEG recording post intervention.

EEG changes

When looking at pre-post tACS changes in EEG oscillatory activity, a trend for an increase in spectral power of gamma oscillations was found (Fig. 3A), with a stronger effect for oscillatory activity around the stimulation frequency (i.e., stim γ=38–42 Hz; Mann-Whitney U= 3,critical U=2, p = 0.186).Analysis of daily EEG recordings indexing changes of gamma spectral power after each tACS session showed a pattern of increase in spectral power throughout each week of stimulation, with a partial reset of oscillatory activity during the weekends when stimulation was not performed (Fig. 3B). Such pattern was visible for stimulation electrodes (P7, P8, T7, T8) but not for control electrodes recording activity from frontal (AFz, F1, F2) and centro-parietal(C3, C4, Pz) regions of the brain.

P-Tau imaging

[^{18}F]-FTPPET revealed supra-threshold decreases of intracerebral p-Tau burden in temporal lobe regions targeted by tACS following the tACS treatment in 3/4 patients (Sbj #1 = 2.0%; Sbj #2 = –5.4%; Sbj #3 = –2.42%; Sbj #4 = –3.58%) (Fig. 3C, D). When looking closer at the sub-regions of the temporal lobe targeted by tACS and relevant for AD (i.e., mesial structures such as entorhinal cortex and parahippocampus) a group-level [^{18}F]-FTP PET binding decrease after the tACS treatment was observed (Supplementary Figure 1).

Microglia imaging

One subject displayed a significant decrease of microglia activation (Sbj #2 = –6.19%), paralleled by a significant decrease of p-Tau burden after the intervention (Sbj #2 = –5.4%). In the other 3 participants, changes were not significant (Sbj #1 = 2.30%; Sbj #3 = –0.38; Sbj #4 = 1.57) (Fig. 3C, D). However, when analyzing the group average changes on the stimulated temporal regions, a trend toward a decrease was found for the same mesial structures observed when analyzing p-Tau changes (Supplementary Figure 1).

Aβ imaging

[^{11}C]-PiB binding did not show any significant longitudinal changes considering that relative measurement error of the test-retest of Aβ-PET can reach 3–5% (Sbj #1 = –2.21%; Sbj #2 = –1.27%; Sbj #3 = –1.74%; Sbj #4 = 0.76%) (Fig. 3C).

Cognitive assessment

No significant changes in overall cognition were found after tACS using a Mann-Whitney nonparametric *U* test (ADAS-Cog baseline mean = 22.75, SD=8.2, post = 24.6, SD = 11.4, Mann-Whitney $U = 12$, critical $U = 2$, p.= 0.745; ADL baseline mean = 70, SD = 2.6, post = 70, SD = 3.5, Mann-Whitney $U = 12$, critical $U = 2$, $p. = 0.842$; MMSE baseline mean = 20.7, SD = 3.6, post = 20.5, SD = 3.4, Mann-Whitney $U = 12$, critical $U = 2$, $p. = 0.813$; MoCA: baseline mean = 13.25, SD = 4.1, post = 13.5, SD = 5.8, Mann-Whitney $U = 9$, critical $U = 2$, $p. = 0.725$), establishing safety of the tACS intervention.

DISCUSSION

Preliminary data from our case series suggest that 20 hours of daily gamma tACS distributed across 1 month is safe and tolerable in AD patients. A trend toward increase in gamma spectral power was detected after the tACS intervention and 3/4 patients showed a decrease in p-Tau burden over the stimulated temporal lobe regions (Fig. 3). Pilot results are consistent with results obtained via optogenetic and sensory stimulation in the gamma band in preclinical models of AD [42]. However, we did not observe the originally predicted change in Aβ load based on the aforementioned results in a mouse model of AD. Considering the typical pattern of Aβ and p-Tau deposition in the human brain when patients are symptomatic—i.e., a diffuse Aβ pathology over the temporal, parietal, and prefrontal cortex in contrast with a p-Tau spread mostly affecting the temporal lobes (Fig. 2), we can speculate that gamma entrainment could be more effective on p-Tau rather than Aβ load in our study based on the specific tACS montage adopted [43]. In any case, we cannot completely rule out the possibility that the observed p-Tau burden decrease is due to intra-subject variability, even if thresholds for test-retest significance were defined according to most recent PET reliability literature. Further investigations on a bigger population are needed in order to determine the replicability of those current results since test-retest PET reliability thresholds have been determined based on studies with similar but not identical conditions.

Microglia show a neuroprotective role under physiological conditions [44], and there is mounting evidence that neuroinflammation plays a critical role in the pathophysiology of

AD [45]. In AD, microglia has been suggested to play a dual role, that is a positive effect on protein clearance when acutely activated, and a neurotoxic and pro-inflammatory one when chronically activated [46]. Even though the decrease observed in Sbj #3 could be in line with tACS reducing chronic hyperactivation in advanced AD patients, the lack of changes in the rest of the sample, as well as the non-significant changes in A, could also be explained by a non-optimal window of observation, i.e., acutely after the intervention. Timing of sampling post-intervention is a key parameter to evaluate the impact of gamma-induction protocols and should be carefully considered in future studies. Regardless, the microglia-amyloid-tau crosstalk is still under investigation and clear pathophysiological cascades have not been identified yet. Interestingly, p-Tau and Aβ seem to colocalize in only the 0.02% of same synapses in the human brain and, even more intriguingly, microglia have been observed especially in proximity of p-Tau tangles [43]. These observations seem to suggest that microglia could have a more direct and effective action (i.e., clearance) on p-Tau aggregates rather than Aβ plaques because of this preferential colocalization. Furthermore, the PBR28 tracer has some limitations. TSPO is expressed throughout the brain, making the identification of a suitable reference region in PET analysis problematic. Also, the tracer seems to bind the pro-inflammatory phenotype of microglia (so called M1) but to some extent also the anti-inflammatory phenotype of microglia (called M2) [47]. The lack of a specific phenotype binding could have limited the detection of microglia activation changes in our sample. Overall, data suggest microglia PET imaging able to capture neuroinflammation in the temporal lobe in AD patients and promote its use for longitudinal evaluations of gamma-inducing protocols in AD.

The present study was conducted as a proof-of-principle of safety and feasibility in translating gamma-induction protocols used in animal models of AD to actual AD patients, including outcome measures related to protein clearance and neuroinflammation. The small sample was mostly due to the burden of multiple ($n = 6$) PET scans repeated over time and the long tACS treatment (average tACS exposure in literature is approximately 1 h per study, versus 20 h in the present protocol), and the study was not designed to show significant cognitive changes over a short 4-week window while at the same time providing valuable insight on the safety of sustained tACS delivery in AD patients. Given the demonstrated safety and feasibility, the dataset could be leveraged to design larger trials with even longer interventions (potentially in home-based settings), focusing on p-Tau pathology and neuroinflammation, and additional time points for PET imaging and/or blood markers. Importantly, the present pilot results support the safety and feasibility of applying a high dose of tACS (20 hours) for relatively long periods of time to broadly restore brain oscillations in AD. Interestingly, Slow Wave Activity (SWA, present in NREM sleep) as well as slow cortical oscillations (<1Hz) are also impaired in AD, with preliminary evidences on AD mouse models showing how optogenetic restoration of SWA is able to inhibit Aβ accumulation and prevent calcium overload [48, 49]. Similar interventions via tACS could be implemented.

Conclusions

The study demonstrates preliminary evidence of safety, feasibility and potential impact of 40 Hz tACS on protein clearance in mild-to-moderate AD, promoting an interesting avenue of research in the quest for the identification of novel therapeutics for AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors would like to thank patients and caregivers for their support and the Defense Advanced Research Projects Agency (DARPA) for their support to the study via HR001117S0030. Emiliano Santarnecchi is supported by the Beth Israel Deaconess Medical Center (BIDMC) via the Chief Academic Officer (CAO) Award 2017, the DARPA via HR001117S0030, the NIH (P01 AG031720-06A1, R01 MH117063-01, R01 AG060981-01), the Alzheimer's Drug Discovery Foundation (ADDF) and the Association for Frontotemporal Dementia (AFTD) via GA 201902-2017902.

REFERENCES

[1]. Fratiglioni L, Launer LJ, Andersen K, Breteler MM, Copeland JR, Dartigues JF, Lobo A, Martinez-Lage J, Soininen H, Hofman A (2000) Incidence of dementia and major subtypes in Europe: A collaborative study of population based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology 54, S10–15. [PubMed: 10854355]

[2]. Forester BP, Patrick RE, Harper DG (2020) Setbacks and opportunities in disease-modifying therapies in Alzheimer disease. JAMA Psychiatry 77, 7–8. [PubMed: 31532462]

[3]. Bejanin A, Schonhaut DR, La Joie R, Kramer JH, Baker SL, Sosa N, Ayakta N, Cantwell A, Janabi M, Lauriola M, O'Neil JP, Gorno-Tempini ML, Miller ZA, Rosen HJ, Miller BL, Jagust WJ, Rabinovici GD (2017) Tau pathology and neurodegeneration contribute to cognitive impairment in Alzheimer's disease. Brain 140, 3286–3300. [PubMed: 29053874]

[4]. Bartels T, De Schepper S, Hong S (2020) Microglia modulate neurodegeneration in Alzheimer's and Parkinson's diseases. Science 370, 66–69. [PubMed: 33004513]

[5]. Jack CR, Wiste HJ, Lesnick TG, Weigand SD, Knopman DS, Vemuri P, Pankratz VS, Senjem ML, Gunter JL, Mielke MM, Lowe VJ, Boeve BF, Petersen RC (2013) Brain β-amyloid load approaches a plateau. Neurology 80, 890–896. [PubMed: 23446680]

[6]. Pontecorvo MJ, Devous MD, Navitsky M, Lu M, Salloway S, Schaerf FW, Jennings D, Arora AK, McGeehan A, Lim NC, Xiong H, Joshi AD, Siderowf A, Mintun MA, 18FAV-1451-A05 investigators (2017) Relationships between flortaucipir PET tau binding and amyloid burden, clinical diagnosis, age and cognition. Brain 140, 748–763. [PubMed: 28077397]

[7]. Sanchez JS, Becker JA, Jacobs HIL, Hanseeuw BJ, Jiang S, Schultz AP, Properzi MJ, Katz SR, Beiser A, Satizabal CL, O'Donnell A, DeCarli C, Killiany R, El Fakhri G, Normandin MD, Gómez-Isla T, Quiroz YT, Rentz DM, Sperling RA, Seshadri S, Augustinack J, Price JC, Johnson KA (2021) The cortical origin and initial spread of medial temporal tauopathy in Alzheimer's disease assessed with positron emission tomography. Sci Transl Med 13, eabc0655.

[8]. Palop JJ, Mucke L (2010) Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: From synapses toward neural networks. Nat Neurosci 13, 812–818. [PubMed: 20581818]

[9]. Babiloni C, Lizio R, Marzano N, Capotosto P, Soricelli A, Triggiani AI, Cordone S, Gesualdo L, Del Percio C (2016) Brain neural synchronization and functional coupling in Alzheimer's disease as revealed by resting state EEG rhythms. Int J Psychophysiol 103, 88–102. [PubMed: 25660305]

[10]. Palop JJ, Mucke L (2016) Network abnormalities and interneuron dysfunction in Alzheimer disease. Nat Rev Neurosci 17, 777–792. [PubMed: 27829687]

[11]. Iaccarino HF, Singer AC, Martorell AJ, Rudenko A, Gao F, Gillingham TZ, Mathys H, Seo J, Kritskiy O, Abdurrob F, Adaikkan C, Canter RG, Rueda R, Brown EN, Boyden ES, Tsai L-H

(2016) Gamma frequency entrainment attenuates amyloid load and modifies microglia. Nature 540, 230–235. [PubMed: 27929004]

[12]. Adaikkan C, Middleton SJ, Marco A, Pao P-C, Mathys H, Kim DN- W, Gao F, Young JZ, Suk H-J, Boyden ES, McHugh TJ, Tsai L-H (2019) Gamma entrainment binds higher-order brain regions and offers neuroprotection. Neuron 102, 929–943.e8. [PubMed: 31076275]

[13]. Thomson H (2018) How flashing lights and pink noise might banish Alzheimer's, improve memory and more. Nature 555, 20–22.

[14]. Reed T, Cohen Kadosh R (2018) Transcranial electrical stimulation (tES) mechanisms and its effects on cortical excitability and connectivity. J Inherit Metab Dis 41, 1123–1130.

[15]. Santarnecchi E, Sprugnoli G, Bricolo E, Costantini G, Liew S-L, Musaeus CS, Salvi C, Pascual-Leone A, Rossi A, Rossi S (2019) Gamma tACS over the temporal lobe increases the occurrence of Eureka! moments. Sci Rep 9, 5778. [PubMed: 30962465]

[16]. Santarnecchi, Polizzotto NR, Godone M, Giovannelli, Feurra M, Matzen L, Rossi A, Rossi S (2013) Frequencydependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. Curr Biol 23, 1449–1453. [PubMed: 23891115]

[17]. Jack CR, Wiste HJ, Botha H, Weigand SD, Therneau TM, Knopman DS, Graff-Radford J, Jones DT, Ferman TJ, Boeve BF, Kantarci K, Lowe VJ, Vemuri P, Mielke MM, Fields JA, Machulda MM, Schwarz CG, Senjem ML, Gunter JL, Petersen RC (2019) The bivariate distribution of amyloid-β and tau: Relationship with established neurocognitive clinical syndromes. Brain 142, 3230–3242. [PubMed: 31501889]

[18]. Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, Szoeke C, Macaulay SL, Martins R, Maruff P, Ames D, Rowe CC, Masters CL, Australian Imaging Biomarkers and Lifestyle (AIBL) Research Group (2013) Amyloid deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: A prospective cohort study. Lancet Neurol 12, 357–367. [PubMed: 23477989]

[19]. Frohlich F, McCormick DA (2010) Endogenous electric fields may guide neocortical network activity. Neuron 67, 129–143. [PubMed: 20624597]

[20]. Tatti E, Rossi S, Innocenti I, Rossi A, Santarnecchi E (2016) Non-invasive brain stimulation of the aging brain: State of the art and future perspectives. Ageing Res Rev 29, 66–89. [PubMed: 27221544]

[21]. Nedergaard M, Goldman SA (2020) Glymphatic failure as a final common pathway to dementia. Science 370, 50–56. [PubMed: 33004510]

[22]. Santarnecchi, Muller, Rossi S, Sarkar A, Polizzotto NR, Rossi A, Cohen Kadosh R (2016) Individual differences and specificity of prefrontal gamma frequency-tACS on fluid intelligence capabilities. Cortex 75, 33–43. [PubMed: 26707084]

[23]. Santarnecchi E, Biasella A, Tatti E, Rossi A, Prattichizzo D, Rossi S (2017) High-gamma oscillations in the motor cortex during visuo-motor coordination: A tACS interferential study. Brain Res Bull 131, 47–54. [PubMed: 28322886]

[24]. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergström M, Savitcheva I, Huang G, Estrada S, Ausén B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Långström B (2004) Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol 55, 306–319. [PubMed: 14991808]

[25]. Johnson KA, Schultz A, Betensky RA, Becker JA, Sepulcre J, Rentz D, Mormino E, Chhatwal J, Amariglio R, Papp K, Marshall G, Albers M, Mauro S, Pepin L, Alverio J, Judge K, Philiossaint M, Shoup T, Yokell D, Dickerson B, Gomez-Isla T, Hyman B, Vasdev N, Sperling R (2016) Tau positron emission tomographic imaging in aging and early Alzheimer disease. Ann Neurol 79, 110–119. [PubMed: 26505746]

[26]. Kreisl WC, Lyoo CH, McGwier M, Snow J, Jenko KJ, Kimura N, Corona W, Morse CL, Zoghbi SS, Pike VW, McMahon FJ, Turner RS, Innis RB, Biomarkers Consortium PET Radioligand Project Team (2013) *In vivo* radioligand binding to translocator protein correlates with severity of Alzheimer's disease. Brain 136, 2228–2238. [PubMed: 23775979]

[27]. Rosen WG, Mohs RC, Davis KL (1984) A new rating scale for Alzheimer's disease. Am J Psychiatry 141, 1356–1364. [PubMed: 6496779]

[28]. Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12, 189–198. [PubMed: 1202204]

[29]. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, Cummings JL, Chertkow H (2005) The Montreal Cognitive Assessment, MoCA: A brief screening tool for mild cognitive impairment. J Am Geriatr Soc 53, 695–699. [PubMed: 15817019]

[30]. Galasko D, Bennett D, Sano M, Ernesto C, Thomas R, Grundman M, Ferris S (1997) An inventory to assess activities of daily living for clinical trials in Alzheimer's disease. The Alzheimer's Disease Cooperative Study. Alzheimer Dis Assoc Disord 11(Suppl 2), S33–39. [PubMed: 9236950]

[31]. Wessels AM, Lines C, Stern RA, Kost J, Voss T, Mozley LH, Furtek C, Mukai Y, Aisen PS, Cummings JL, Tariot PN, Vellas B, Dupre N, Randolph C, Michelson D, Andersen SW, Shering C, Sims JR, Egan MF (2020) Cognitive outcomes in trials of two BACE inhibitors in Alzheimer's disease. Alzheimers Dement 16, 1483–1492. [PubMed: 33049114]

[32]. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC,´ Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS, Killiany RJ(2006) An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 31, 968–980. [PubMed: 16530430]

[33]. Baker SL, Maass A, Jagust WJ (2017) Considerations and code for partial volume correcting [18F]-AV-1451 tau PET data. Data Brief 15, 648–657. [PubMed: 29124088]

[34]. McNamee RL, Yee S-H, Price JC, Klunk WE, Rosario B, Weissfeld L, Ziolko S, Berginc M, Lopresti B, Dekosky S, Mathis CA (2009) Consideration of optimal time window for Pittsburgh compound B PET summed uptake measurements. J Nucl Med 50, 348–355. [PubMed: 19223409]

[35]. Lyoo CH, Ikawa M, Liow J-S, Zoghbi SS, Morse CL, Pike VW, Fujita M, Innis RB, Kreisl WC (2015) Cerebellum can serve as a pseudo-reference region in Alzheimer disease to detect neuroinflammation measured with PET radioligand binding to translocator protein. J Nucl Med 56, 701–706. [PubMed: 25766898]

[36]. Lopresti BJ, Klunk WE, Mathis CA, Hoge JA, Ziolko SK, Lu X, Meltzer CC, Schimmel K, Tsopelas ND, DeKosky ST, Price JC (2005) Simplified quantification of Pittsburgh Compound B amyloid imaging PET studies: A comparative analysis. J Nucl Med 46, 1959–1972. [PubMed: 16330558]

[37]. Hanseeuw BJ, Betensky RA, Jacobs HIL, Schultz AP, Sepulcre J, Becker JA, Cosio DMO, Farrell M, Quiroz YT, Mormino EC, Buckley RF, Papp KV, Amariglio RA, Dewachter I, Ivanoiu A, Huijbers W, Hedden T, Marshall GA, Chhatwal JP, Rentz DM, Sperling RA, Johnson K (2019) Association of amyloid and tau with cognition in preclinical Alzheimer disease: A longitudinal study. JAMA Neurol 76, 915–924. [PubMed: 31157827]

[38]. Bullich S, Seibyl J, Catafau AM, Jovalekic A, Koglin N, Barthel H, Sabri O, De Santi S (2017) Optimized classification of 18F-Florbetaben PET scans as positive and negative using an SUVR quantitative approach and comparison to visual assessment. Neuroimage Clin 15, 325–332. [PubMed: 28560157]

[39]. Devous MD, Joshi AD, Navitsky M, Southekal S, Pontecorvo MJ, Shen H, Lu M, Shankle WR, Seibyl JP, Marek K, Mintun MA (2018) Test-retest reproducibility for the tau PET imaging agent Flortaucipir F 18. J Nucl Med 59, 937–943. [PubMed: 29284675]

[40]. Heeman F, Hendriks J, Lopes Alves I, Ossenkoppele R, Tolboom N, van Berckel BNM, Lammertsma AA, Yaqub M, AMYPAD Consortium (2020) [11C]PIB amyloid quantification: Effect of reference region selection. EJNMMI Res 10, 123. [PubMed: 33074395]

[41]. Nair A, Veronese M, Xu X, Curtis C, Turkheimer F, Howard R, Reeves S (2016) Test-retest analysis of a noninvasive method of quantifying [(11)C]-PBR28 binding in Alzheimer's disease. EJNMMI Res 6, 72. [PubMed: 27678494]

[42]. Martorell AJ, Paulson AL, Suk H-J, Abdurrob F, Drummond GT, Guan W, Young JZ, Kim DN-W, Kritskiy O, Barker SJ, Mangena V, Prince SM, Brown EN, Chung K, Boyden ES, Singer AC, Tsai L-H (2019) Multi-sensory gamma stimulation ameliorates Alzheimer's-associated pathology and improves cognition. Cell 177, 256–271.e22. [PubMed: 30879788]

[43]. Busche MA, Hyman BT (2020) Synergy between amyloid-β and tau in Alzheimer's disease. Nat Neurosci 23, 1183–1193. [PubMed: 32778792]

[44]. Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. Science 308, 1314–1318. [PubMed: 15831717]

[45]. Leng F, Edison P (2021) Neuroinflammation and microglial activation in Alzheimer disease: Where do we go from here? Nat Rev Neurol 17, 157–172. [PubMed: 33318676]

[46]. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Munch AE, Chung W-¨S, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA(2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541, 481–487. [PubMed: 28099414]

[47]. Liu B, Le KX, Park M-A, Wang S, Belanger AP, Dubey S, Frost JL, Holton P, Reiser V, Jones PA, Trigg W, Di Carli MF, Lemere CA (2015) *In vivo* detection of age- and disease-related increases in neuroinflammation by 18F-GE180 TSPO microPET imaging in wild-type and Alzheimer's transgenic mice. J Neurosci 35, 15716–15730.

[48]. Kastanenka KV, Hou SS, Shakerdge N, Logan R, Feng D, Wegmann S, Chopra V, Hawkes JM, Chen X, Bacskai BJ (2017) Optogenetic restoration of disrupted slow oscillations halts amyloid deposition and restores calcium homeostasis in an animal model of Alzheimer's disease. PLoS One 12, e0170275.

[49]. Lee YF, Gerashchenko D, Timofeev I, Bacskai BJ, Kastanenka KV (2020) Slow wave sleep is a promising intervention target for Alzheimer's disease. Front Neurosci 14, 705. [PubMed: 32714142]

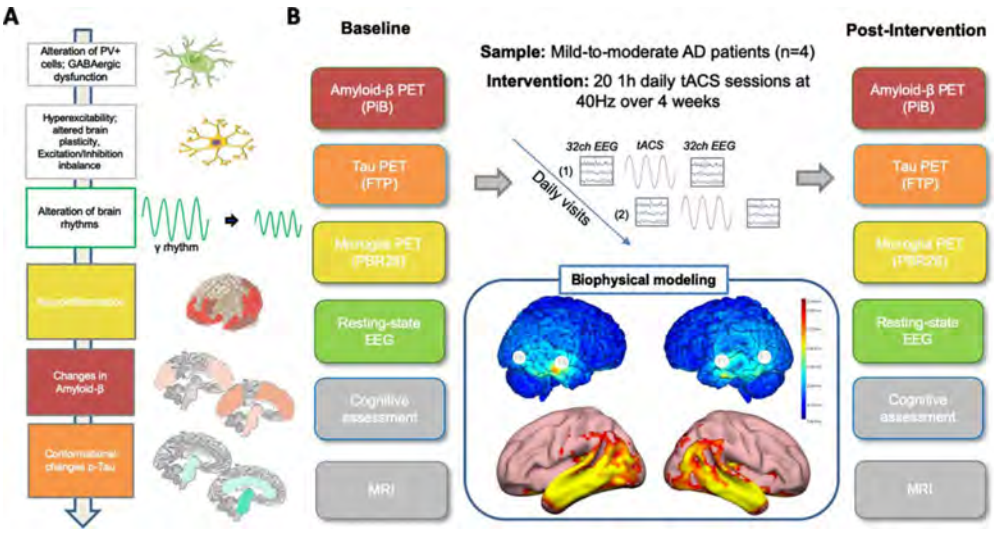

Fig. 1.

Study Design. A) A simplified conceptual framework of the neuropathophysiology of AD is shown, describing the potential cascade involving interneuron dysfunction, altered excitation/inhibition balance, alterations of brain rhythms, neuroinflammatory response and protein accumulation. B) An attempt to map the different levels of the model directly in AD patients was made in the study design, with repeated EEG, amyloid-β, p-Tau and microglia PET imaging before and after the tACS treatment course. Neuroimaging and cognitive data were collected as well. Biophysical modeling displays the focus on bilateral temporal lobes and the resulting induced electrical field closely resembling p-Tau and amyloid-β accumulation.

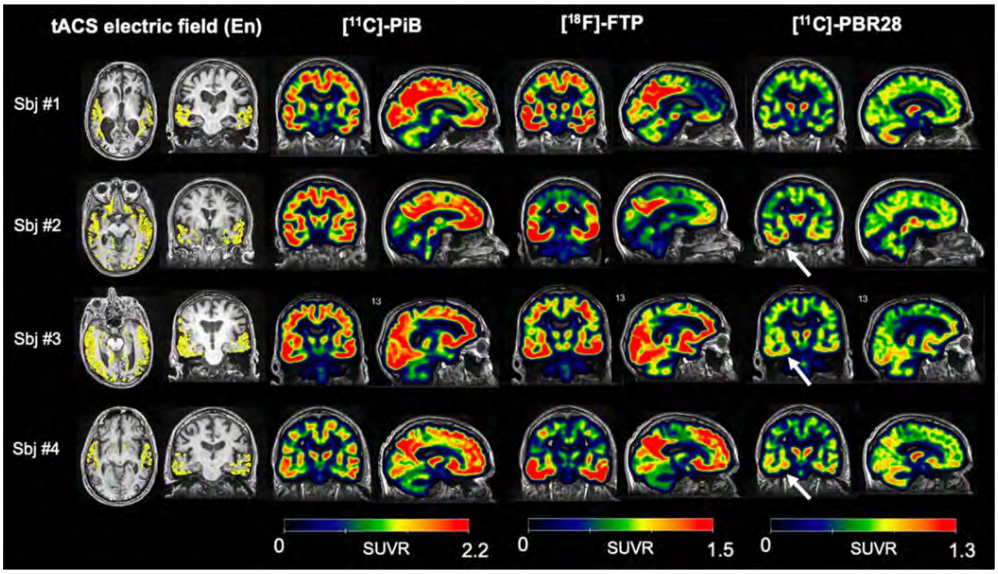

Fig. 2.

tACS targets and SUVr parametric images for all participants. Axial and coronal views of the normal electric field induced by tACS in each participant are reported (yellow, En > 0.25 V/m on the structural MRI for anatomical reference), showing the tACS field affecting primarily the bilateral temporal lobes (left). Individual sagittal and coronal views of SUVr maps of [^{11}C]-PiB, [^{18}F]-FTP and [^{11}C]-PBR28 data collected at baseline for each patient are shown, displaying high levels of amyloid-β in all the participants, as well as significant p-Tau accumulation and signs of neuroinflammation (white arrows) in the bilateral temporal lobes, thalamus and parietal regions. Note: images are shown in radiological convention.

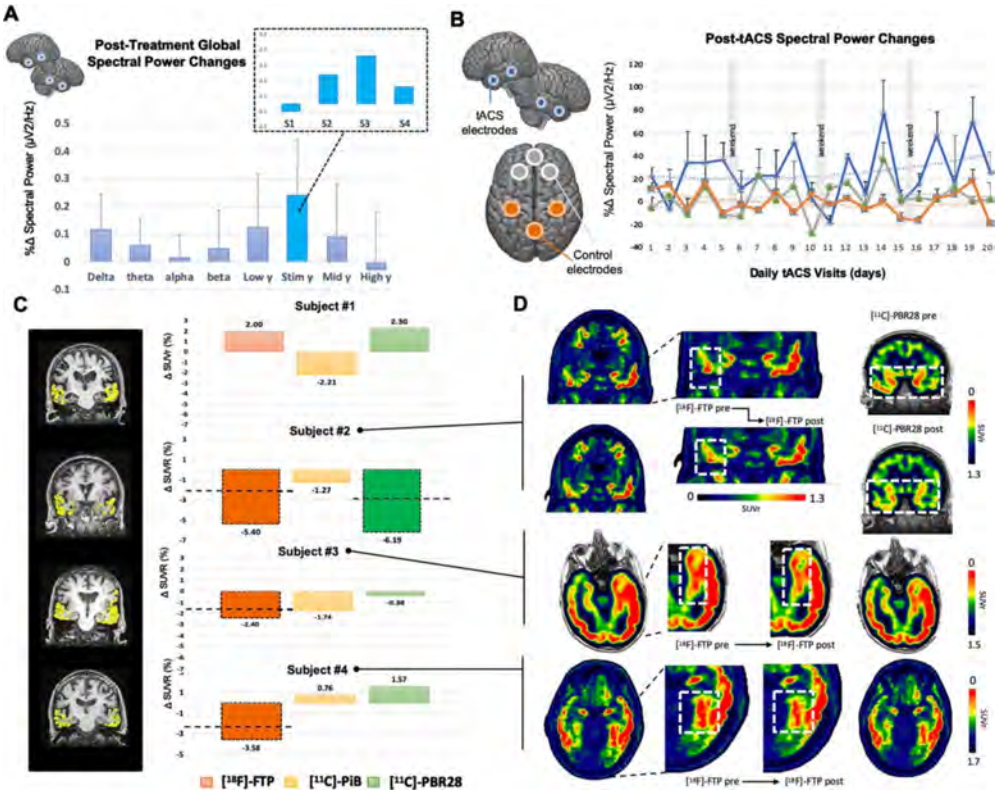

Fig. 3.

Results. A) Participants reported a trend for an increase in spectral power of gamma oscillations, with a stronger effect for activity around the stimulation frequency (i.e., stim γ at 38–42 Hz). B) Daily EEG recordings before and after each tACS session showed an incremental effect of gamma spectral power over the stimulation electrodes placed on the temporal lobes (T8, P8, T7, P7), and no apparent changes in control electrodes indexing activity in frontal (Afz, F1, F2) and centro-parietal (C3, C4, Pz) regions. C) Delta SUVr between baseline and follow up are reported for the three PET tracers and specifically for SUVr values extracted from the individual tACS stimulation maps based on biophysical modeling (yellow, stimulation higher than 0.25 V/m). A significant (>1.98%) change in p-Tau deposition was observed for 3 patients (#2, #3, #4), as well as a decrease in microglia activation after tACS for patient #2. D) Individual data for participant #2, #3, and #4 showing example regions of putative change in p-Tau and microglia SUVr after tACS.

Table 1

ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale; BDNF, brain-derived neurotrophic factor; F, female; M, male; MMSE, Mini-Mental State Examination

Study ID	Age	Sex	Dominant Hand	Years of Education	Race	Ethnicity	MMSE baseline	ADAS-Cog baseline	*APOE* status	BDNF polymorphism
Sbj 001	84	M	Right	Master's Degree	White	Non-Hispanic	18	29	*ε3/ε3*	Val/Val
Sbj 002	73	F	Right	Master's Degree	White	Non-Hispanic	19	30	*ε4/ε4*	Val/Met
Sbj 003	84	M	Left	PhD	White	Non-Hispanic	20	14	*ε3/ε4*	Val/Val
Sbj 004	64	M	Right	Master's Degree	White	Non-Hispanic	26	18	*ε4/ε4*	Val/Val

RESEARCH ARTICLE

Increasing Brain Gamma Activity Improves Episodic Memory and Restores Cholinergic Dysfunction in Alzheimer's Disease

Alberto Benussi, MD [1,2] Valentina Cantoni, MS,[1] Mario Grassi, PhD,[3] Lucie Brechet, PhD,[4] Christoph M. Michel, MD, PhD,[4,5] Abhishek Datta, MS, PhD,[6] Chris Thomas, MS,[6] Stefano Gazzina, MD,[7] Maria Sofia Cotelli, MD,[8] Marta Bianchi, MD,[8] Enrico Premi, MD,[9] Yasmine Gadola, MS,[1] Maria Cotelli, MS, PhD,[10] Marta Pengo, MD,[11] Federica Perrone, PhD,[1] Maria Scolaro, MS,[7] Silvana Archetti, BS,[12] Eino Solje, MD, PhD [13,14] Alessandro Padovani, MD, PhD,[1,2] Alvaro Pascual-Leone, MD, PhD [15,16,17] and Barbara Borroni, MD [1,2]

Objective: This study aimed to assess whether non-invasive brain stimulation with transcranial alternating current stimulation at gamma-frequency (γ-tACS) applied over the precuneus can improve episodic memory and modulate cholinergic transmission by modulating cerebral rhythms in early Alzheimer's disease (AD).
Methods: In this randomized, double-blind, sham controlled, crossover study, 60 AD patients underwent a clinical and neurophysiological evaluation including assessment of episodic memory and cholinergic transmission pre and post 60 minutes treatment with γ-tACS targeting the precuneus or sham tACS. In a subset of 10 patients, EEG analysis and individualized modelling of electric field distribution were carried out. Predictors to γ-tACS efficacy were evaluated.
Results: We observed a significant improvement in the Rey Auditory Verbal Learning (RAVL) test immediate recall ($p < 0.001$) and delayed recall scores ($p < 0.001$) after γ-tACS but not after sham tACS. Face-name associations scores improved with γ-tACS ($p < 0.001$) but not after sham tACS. Short latency afferent inhibition, an indirect measure of cholinergic transmission, increased only after γ-tACS ($p < 0.001$). *ApoE* genotype and baseline cognitive impairment were the best predictors of response to γ-tACS. Clinical improvement correlated with the increase in gamma frequencies in posterior regions and with the amount of predicted electric field distribution in the precuneus.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.26411

Received Oct 18, 2021, and in revised form May 13, 2022. Accepted for publication May 16, 2022.

Address correspondence to Dr Borroni, Clinica Neurologica, Università degli Studi di Brescia, P.le Spedali Civili 1, 25123, Brescia, Italy.
E-mail: bborroni@inwind.it

From the [1]Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy; [2]Neurology Unit, Department of Neurological and Vision Sciences, ASST Spedali Civili, Brescia, Italy; [3]Department of Brain and Behavioural Sciences, Medical and Genomic Statistics Unit, University of Pavia, Pavia, Italy; [4]Functional Brain Mapping Laboratory, Department of Fundamental Neuroscience, University of Geneva, Geneva, Switzerland; [5]Center for Biomedical Imaging (CIBM), Lausanne, Switzerland; [6]Research & Development, Soterix Medical, Inc., New York, USA; [7]Neurophysiology Unit, Department of Neurological and Vision Sciences, ASST Spedali Civili, Brescia, Italy; [8]Neurology Unit, Valle Camonica Hospital, Brescia, Italy; [9]Stroke Unit, Department of Neurological and Vision Sciences, ASST Spedali Civili, Brescia, Italy; [10]Neuropsychology Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy; [11]Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; [12]Clinical Chemistry Laboratory, Diagnostic Department, ASST Spedali Civili Brescia, Brescia, Italy; [13]Institute of Clinical Medicine, Neurology, University of Eastern Finland, Kuopio, Finland; [14]Neuro center, Neurology, Kuopio University Hospital, Kuopio, Finland; [15]Department of Neurology, Harvard Medical School, Boston, Massachusetts, USA; [16]Hinda and Arthur Marcus Institute for Aging Research and Deanna and Sidney Wolk Center for Memory Health, Hebrew SeniorLife, Boston, MA, USA; and [17]Guttmann Brain Health Institut, Barcelona, Spain

Additional supporting information can be found in the online version of this article.

Interpretation: Precuneus γ-tACS, able to increase γ-power activity on the posterior brain regions, showed a significant improvement of episodic memory performances, along with restoration of intracortical excitability measures of cholinergic transmission. Response to γ-tACS was dependent on genetic factors and disease stage.

ANN NEUROL 2022;92:322–334

The World Health Organization has declared Alzheimer's disease (AD) a priority health problem, due to its increasing incidence and high societal impact, and there is an urgent need for the identification of novel therapeutic targets.[1]

Cholinergic enhancement has been the mainstay of AD therapeutics from 1996 up to now,[2] and very recently, aducanumab, which reduces beta amyloid plaques deposition,[3] has received Food and Drug Administration approval.[4]

Along with cholinergic deficits and amyloid deposition as pathological hallmarks of AD, recent literature has highlighted gamma desynchronization as an early occurrence, thus holding the potential to be used as an additional therapeutic target. In particular, AD is characterized by a prominent disruption of oscillations in the gamma frequency band (30–80 Hz), which is proportional to disease severity and progression.[5, 6]

The clinical potential of restoring gamma oscillations via noninvasive brain stimulation has recently gained attention. Indeed, restoration of gamma oscillations by neural entrainment in animal models of AD induces a remarkable decrease in the pathological burden of amyloid and significantly improves cognitive performance.[7–9]

In this context, transcranial alternating current stimulation (tACS) is a unique noninvasive tool which allows the modulation of brain rhythms at specific frequencies in pre-defined cerebral regions,[10] and that can result in improvement of cognitive processes in healthy subjects.[11–14] tACS is easy to apply, safe, painless, inexpensive and deliverable in at-home settings.[15] In a pilot study carried out in patients with mild dementia due to AD, we demonstrated that exposure to γ-tACS targeting the precuneus led to a significant improvement in performance in several memory tasks, along with the restoration of intracortical excitability measures of cholinergic neurotransmission, compared to sham-tACS.[16] Despite the promising results, several issues needed still to be addressed, and were the objective of the present study: (a) to confirm and extend in an independent larger sample of subjects the efficacy of γ-tACS over the precuneus on episodic memory performances and cholinergic neurotransmission; (b) to demonstrate that γ-tACS can modulate brain activity and that the predicted enhancement of gamma activity accounted for (or at least was associated with) the memory effects; and (c) to assess possible predictors of response to the γ-tACS intervention, such as *apolipoprotein E (ApoE)* genotype, the major recognized genetic risk factor for late-onset AD,[17] and *brain derived neurotrophic factor (BDNF)* genotype, previously shown to affect transcranial stimulation effectiveness.[18, 19]

Methods

Participants

Participants fulfilling current criteria for AD[20] were recruited at the Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy.

At enrollment, each patient underwent a standardized neuropsychological assessment, as previously published,[21] a structural imaging study, and a blood sampling for genotype analyses. Cognitive reserve was evaluated with the Italian version of the Cognitive Reserve Index (CRIq) which is based on education levels, working activities, and leisure time.[22]

AD diagnosis was corroborated by either cerebrospinal fluid (CSF) analysis supporting an AD pathological process ($A\beta_{1-42} \leq 600$ ng/L and tau≥400 ng/L) or positive amyloid-positron emission tomography (PET) scan.[23]

The following exclusion criteria were applied: (a) cerebrovascular disorders, hydrocephalus, and intracranial mass documented by magnetic resonance imaging (MRI); (b) history of traumatic brain injury; (c) serious medical illness other than AD; (d) history of seizures; (e) metal implants in the head; (f) electronic implants (i.e., pacemaker).

Participants who were already on a pharmacologic regimen were allowed to continue it provided it had been unchanged for 6 weeks prior to the intervention, but initiation of drugs after the start of the observation period was not allowed.

Full written informed consent was obtained from all participants according to the Declaration of Helsinki. The study protocol was approved by the local ethics committee (Brescia Hospital, #NP4479). The trial was registered at ClinicalTrials.gov (NCT04842955).

Study Design

For the main study, participants were randomized into two groups in a 1:1 ratio and each group received a single session of exposure to γ-tACS targeting the precuneus or a

single session of sham-tACS first and, after 1 week, stimulation was inverted (crossover phase) (see Supporting Information Figure S1, which is available online). In each session, a set of tasks assessing episodic memory was tested twice, at baseline (pre-stimulation) and after tACS (post-stimulation). Moreover, a memory task assessing associative memory was carried out during the last 20 minutes of tACS stimulation (see neuropsychological assessment below). In each session, a transcranial magnetic stimulation (TMS) protocol assessing short-latency afferent inhibition (SAI), an indirect measure of cholinergic transmission, was tested twice in all subjects, at baseline (pre-stimulation) and after tACS (post-stimulation) (see TMS assessment below). In a subset of 10 participants, an electroencephalogram (EEG) was recorded twice in each session, at baseline (pre-stimulation) and immediately after tACS (post-stimulation), before TMS assessment (see EEG assessment below).

We performed two supporting information studies in order to assess the specific effects of γ-tACS on other cognitive domains (executive functions, verbal fluency and visuospatial abilities - supporting information study 1) and site (regarding the specific stimulated brain region - supporting information study 2) (see Supporting Information Figure S1).

For supporting information study 1, 12 participants were randomized into two groups in a 1:1 ratio and each group received a single session of exposure to γ-tACS targeting the precuneus or a single session of sham-tACS first and, after 1 week, stimulation was inverted (crossover phase). In each session, a set of tasks assessing executive functions, verbal fluency and visuospatial abilities (digit span backward, phonemic and semantic fluencies, trail making test part A and B, clock drawing test) was tested twice, at baseline (pre-stimulation) and after tACS (post-stimulation).

For supporting information study 2, 12 participants were randomized into two groups in a 1:1 ratio and each group received a single session of exposure to γ-tACS targeting the right dorsolateral prefrontal cortex (rDLPFC) or a single session of sham-tACS first and, after 1 week, stimulation was inverted (crossover phase). In each session, the same set of memory tasks performed in the main study was tested twice, at baseline (pre-stimulation) and after tACS (post-stimulation).

Sample size for both supporting information studies was calculated based on results of the main study, to obtain a minimum set of participants in whom significant effects could be observed by assessing each specific cognitive test.

The participants and the examiners performing clinical ratings, tACS, EEG and TMS protocols were blinded to the type of stimulation. B.B. was responsible for random allocation sequences, enrollment of participants, allocation concealment, and assignment of participants to specific interventions. Computer-assisted block randomization was used to randomize subjects into groups that resulted in equal sample sizes.

According to literature data, the effects of a single session of tACS are expected to last for 30–70 minutes.[24] Hence, participants were expected to return to their initial clinical status between the two stimulation sessions which were separated by at least a week.

Outcome Measures

The primary endpoints were a priori defined as: (a) the change from baseline in episodic and associative memory scores after γ-tACS, compared to after sham stimulation; and (b) change from the baseline in gamma brain activity after γ-tACS, compared to after sham stimulation.

The secondary endpoints were defined as: (a) changes from baseline in cholinergic transmission, evaluated indirectly with TMS, and (b) differences in γ-tACS effects on memory performance according to potential predictors, such as *ApoE* and *BDNF* genotypes, along with demographic and clinical covariates.

Cognitive Assessment

To assess the effect of tACS on episodic memory, at each time-point (pre-stimulation and post-stimulation) of γ-tACS targeting the precuneus and sham-tACS, the Rey Auditory Verbal Learning (RAVL) test was carried out, and total recall and long delayed recall were considered.[25] Different lists were randomized and used during pre- and post-stimulation to avoid learning effects.

The Face-Name Association memory Task (FNAT) was used to assess the patient's associative memory and was composed of encoding and retrieval phases.[16, 26] Subjects were seated in a dimly lit room, facing a computer monitor that was placed 60 cm from the subject. The stimuli were presented using Presentation software (Version 14.9, www.neurobs.com) running on a personal computer with a 27-inch screen. During the encoding phase, the patient was shown a gray-scale picture of a face on a monitor together with a proper name, and the patient was required to tell the researcher whether the face belonged to a woman or a man and was required to encode the face-name association. A set of 20 unfamiliar faces was associated to a set of 20 unfamiliar proper names (10 male, 10 female). During the retrieval phase, the patient was shown a face together with four proper names (the correct name, two previously presented names and one new name), and the patient was asked to associate the correct name with each face. Responses were collected via

a response-box, and the stimuli remained on the screen until the response was made.

EEG Recordings

EEG was performed using a NicoletOne EEG system and was continuously recorded from 19 scalp sites positioned according to the 10–20 International System. The ground electrode was positioned in Fpz, while the reference one was positioned at Cz. The electrode for delivering tACS (Pz) was kept on site during the entire recording, in order to avoid reapplication of the EEG cap after tACS and was thus excluded from the EEG recording. The EEG signals were band-pass filtered at 0–500 Hz and digitized at a sampling rate of 1,024 Hz. Skin/electrode impedance was maintained below 5 kΩ. Resting EEG was recorded for 10 min with eyes closed before and after sham and γ-tACS in two separate sessions at least 1 week apart.

EEG Analysis

EEG was analyzed using the freely available academic software Cartool (https://sites.google.com/site/cartoolcommunity). As a first step, data were re-referenced to Fp1, downsampled to 256 Hz and band-pass filtered between 1 and 100 Hz (Non-causal Butterworth filter). Then, independent component analysis (ICA) was applied to remove eye-movement (eye blinks and saccades).[27] Artifact-free epochs of 2 second duration were then visually selected. A fast Fourier transform (FFT) was then calculated for each channel and each 2 second epoch using a Hanning window and averaged across all epochs. The resulting power values were then averaged across four bands: theta (3–6 Hz), alpha (6–12 Hz), beta (12–20 Hz), and gamma (20–40 Hz).

TMS Assessment

A TMS figure-of-eight coil (each loop diameter 70 mm) connected to a monophasic Magstim Bistim^2 system (Magstim Company, Oxford, UK) was used.[28] Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle through surface Ag/AgCl electrodes placed in a belly-tendon montage and acquired using a Biopac MP-150 electromyograph (BIOPAC Systems Inc., Santa Barbara, USA). The TMS coil was held tangentially over the scalp region corresponding to the primary hand motor area contralateral to the target muscle, with the coil handle pointed 45° posteriorly and laterally to the sagittal plane. The "hot spot" was defined as the scalp location from which magnetic stimulation resulted in motor evoked potentials (MEPs) of greatest amplitude with the minimum stimulation intensity, as previously reported.[29]

SAI was studied using a paired-pulse protocol, employing a conditioning-test design.[30] The test stimulus (TS) was adjusted to evoke an MEP of approximately 1 mV peak-to-peak amplitude, while the conditioning stimulus (CS) consisted of a single pulse (200 μs) of electrical stimulation to the right median nerve at the wrist, using a bipolar electrode with the cathode positioned proximally, at an intensity sufficient to evoke a visible twitch of the thenar muscles. Different interstimulus intervals (ISIs) were assessed (0, $^{+}$4 ms), which were fixed relative to the peak latency of the N20 component of the somatosensory evoked potential of the median nerve. For each ISI, 10 different paired CS-TS and control TS were delivered in all participants in a pseudo randomized sequence, with an inter-trial interval of 5 seconds (±10%).

Audio-visual feedback was provided to ensure muscle relaxation during the entire experiment and trials were discarded if EMG activity exceeded 100 μV prior to TMS stimulus delivery. Less than 5% of trials were discarded for each protocol. All of the participants were capable of following instructions and reaching complete muscle relaxation.

Computational Modeling of Electric Field Distribution

Brain images were collected using a 3 T MRI scanner (Siemens Skyra, Erlangen, Germany), equipped with a circularly polarized transmit–receive coil to acquire 3D magnetization-prepared rapid gradient echo (MPRAGE) T1-weighted scans (repetition time 3,200 ms, echo time 402 ms, matrix 256 × 256, field of view 282 mm, slice thickness 1.10 mm, flip angle 8°). This dataset was fused with a model derived from the Visible Human Project to extend the field of volume to the level of the shoulders to mimic the exact experimental montage. The standard Laplace equation was applied considering volume conduction. While the current direction reverses at regular intervals, with the Pz electrode serving as the anode in one cycle and cathode in the other, the cortical electric field (EF) magnitude plot, which indicates where the current is flowing, does not change. The region corresponding to the precuneus was segmented and individually analyzed.

ApoE *and* BDNF *Genotyping*

Genomic DNA was extracted from whole peripheral blood using Maxwell® 16 Blood DNA Purification Kit with Maxwell® 16 Instrument (both Promega). The regions encompassing both APOE rs429358 and rs7412 and *BDNF* rs6265 polymorphisms were amplified by polymerase chain reaction (PCR) using GoTaq® Hot Start Polymerase (Promega) or Optimase® Polymerase (ADS Biotech). PCR products were purified with Amicon® Ultra 0.5 mL Centrifugal Filters (Merck Millipore). Cycle sequencing was performed with the AB Prism Big Dye Terminator Sequencing kit 3.1 (Life Technologies),

following the manufacturer's instructions. Sequences were subsequently purified using MicroSEQ™ ID Sequencing Clean-up Cartridges (Life Technologies) and then loaded on a 3,500 Genetic Analyzer (Life Technologies). All sequences were analyzed using the Chromas software (Technelysium Pty Ltd).

γ-tACS

A single session of tACS was delivered by a battery-driven current stimulator (BrainStim, EMS, Italy) through a pair of saline-soaked (0.9% NaCl) surface sponge electrodes (5.5 × 6 cm). One electrode was placed on the scalp over the precuneus (with the center over Pz position according to the 10–20 international EEG coordinates) and the other over the right deltoid muscle. This particular montage was chosen after performing computational modelling of electric field distribution and considering a previous study with similar montages, showing that tACS with an extracephalic electrode led to significant entrainment of brain oscillations (reported as phase stability) compared to other cephalic montages.[31] For supporting information study 1, we adopted the same montage, while for supporting information study 2, one electrode was placed on the scalp over the rDLPFC (with the center over F4 position according to the 10–20 international EEG coordinates) and the other over the right deltoid muscle.

The electrodes were secured using elastic gauzes, and the electroconductive gel was applied to electrodes to reduce contact impedance (<5 kΩ for all sessions).

During single session real stimulation, an alternating sinusoidal current of 1.5 mA peak-to-baseline (3.0 mA peak-to-peak, current density: 0.09 mA/cm^2) at a frequency of 40 Hz was applied for 60 minutes. For the sham condition, the electrode placement was the same, but the electric current was ramped down 60 seconds after the beginning of the stimulation to make this condition indistinguishable from the experimental stimulation. To detect differences in the perception of the stimulation, participants were asked whether they thought they received real or sham stimulation at the end of each session, and if they perceived tingling cutaneous sensations or phosphenes/light flickering. Sensations were rated on a scale from 0 to 4, with 0 = no sensations reported, 1 = mild, 2 = moderate, 3 = strong, 4 = very strong sensations reported.

During stimulation (both real and sham), participants were sitting in a comfortable chair in a well-lit and quiet room, keeping their eyes open and asked not to speak or move significantly.

Statistical Analyses

Data are expressed as mean ± standard deviation, unless otherwise stated. Baseline demographic and clinical variables were compared across groups using Student's t-test or Fisher's tests, as appropriate. Cohen's Kappa was run to determine if there was an agreement between the type of sensation perceived and the type of stimulation received. A Wilcoxon signed-rank test was used to evaluate differences in perception of cutaneous sensation during real and sham stimulation.

To assess the effect of exposure to γ-tACS (main study and supporting information studies 1 and 2), the cross-over design was analyzed with a linear mixed-effect model by restricted maximum likelihood (REML), considering baseline values, treatment (sham *vs*. real), block (first session of exposure *vs*. second session of exposure after 1 week) and randomization (sham-real *vs*. real-sham) as fixed effects, and patients as random effects. This was adopted to avoid any potential carry-over effects of stimulation on clinical outcomes.

Changes in γ-tACS efficacy associated with potential predictors were evaluated with linear regression models using improvement of cognitive scores after γ-tACS stimulation, or γ-tACS *vs*. sham tACS, as response variables, and *ApoE* genotype (coded as 0 = no ε4 alleles, 1 = one ε4 allele, 2 = two ε4 alleles) and *BDNF* genotype (coded as 0 = no M alleles, 1 = one or two M alleles), along with demographic and clinical characteristics as covariates.

With regard to EEG analyses, paired t-tests were used to compare post *vs*. pre γ-tACS or sham stimulation. As exploratory analysis, Spearman rank-order correlations were used to assess associations between the improvement in memory scores and the increase in gamma frequencies at specific brain regions. A two-sided p-value<0.05 was considered significant.

Statistical analyses were performed using SPSS version 21 (SPSS, Inc., Chicago, USA).

Data Availability

All data, includeing outcome measure results, study protocol, and statistical analysis plan, will be shared through ClinicalTrials.gov via public access (https://clinicaltrials.gov/ct2/show/NCT04842955).

Results

Participants

Seventy participants were initially screened, with two participants not meeting inclusion criteria because they were carriers of an electronic implant (pacemaker) and two more because of a diagnosis of epilepsy; six were excluded because negative to AD biomarkers (see Supporting Information Figure S2). Sixty participants (mean age ± SD = 72.3 ± 7.0 years; female = 51.7%, mean Mini-Mental State Examination (MMSE) score ± SD = 23.9 ± 4.2; mean disease duration±SD = 3.1 ±

TABLE 1. Memory Scores and TMS Measures Before and After Precuneus γ-tACS or Sham Stimulation

	Sham-tACS		γ-tACS Targeting the Precuneus	
Variable	**Baseline**	**Post tACS**	**Baseline**	**Post tACS**
Memory tasks				
RAVL, immediate recall	20.3 ± 6.5	18.7 ± 6.3	18.4 ± 6.9	25.6 ± 8.4[b,c]
RAVL, delayed recall	1.4 ± 1.5	1.0 ± 1.2	1.5 ± 1.4	2.5 ± 2.1[b,c]
FNAT[a]	5.5 ± 2.4		8.2 ± 3.1[b]	
TMS assessment				
Mean SAI (0, $^{+}$4 ms)	0.83 ± 0.15	0.83 ± 0.14	0.86 ± 0.11	0.50 ± 0.13[b,c]

Results are expressed as mean ± standard deviation.
[a]For FNAT, results are reported during stimulation.
[b]Significant difference compared to sham stimulation.
[c]Significant difference compared to baseline.
tACS = transcranial alternating current stimulation; RAVL = Rey Auditory Verbal Learning test; FNAT = face–name associations task; TMS = transcranial magnetic stimulation; SAI = short-latency afferent inhibition.

2.4 years) were enrolled and randomized to receive γ-tACS or sham stimulation first in a 1:1 ratio (see Supporting Information Table S1 for demographic and clinical characteristics).

All participants completed the study and were included in the final analysis. No tACS-related side effects were observed, and tACS was well tolerated by all participants. Regarding the differences in the participants' perception of the stimulation, there was no statistically significant association between type of stimulation, as assessed by Cohen's Kappa ($\kappa = -0.10$, $p = 0.432$). Moreover, tingling cutaneous sensations were equally perceived in both real and sham conditions ($z = 0.852$, $p = 0.394$ by Wilcoxon signed-rank test), and none of the participants reported phosphenes/light flickering, suggesting that exposure to γ-tACS targeting the precuneus could not be distinguished from sham stimulation.

Effects on Episodic Memory

Results for RAVLT immediate and delayed recall, and FNAT scores, pre- and post-stimulation are reported in Table 1 and Figure 1.

By applying mixed-effect models, we observed a significant effect of treatment (γ-tACS vs sham-tACS) on the RAVLT immediate recall ($p < 0.001$), with an estimate difference of −7.0 (90% confidence interval [CI] = −8.2 to −5.8) points, and on the RAVLT delayed recall ($p < 0.001$), with an estimate difference of −1.6 (90% CI = −2.0 to −1.2) points between treatments. In the FNAT, we observed a significant effect of treatment ($p < 0.001$), with an estimate difference of −2.9 (90% CI = −3.6 to −2.3) points between treatments (see Table 2).

Effects on Executive Functions, Verbal Fluency and Visuospatial Abilities

In supporting information study 1, by applying mixed-effect models, we did not observe any significant effect of treatment on digit span backward ($p = 0.600$), phonemic fluencies ($p = 0.439$), semantic fluencies ($p = 0.814$), clock drawing ($p = 0.984$), or trail making test part A ($p = 0.500$) and B ($p = 0.499$).

Effects of γ-tACS on Stimulation Site

In supporting information study 2, we evaluated if the effects of γ-tACS on cognition were site-specific, and applied rDLPFC γ-tACS compared to sham tACS. Contrary to what observed for precuneus γ-tACS, we did not observe significant effects of treatment (γ-tACS vs sham-tACS) on the RAVLT immediate recall ($p = 0.942$), RAVLT delayed recall ($p = 0.983$), and on FNAT scores ($p = 0.588$).

Effect on Cholinergic Dysfunction

TMS measures of cholinergic inhibition, evaluated with SAI, are reported in Table 1 and Figure 1 at each time point. We observed a significant effect of treatment ($p < 0.001$), with an estimate difference of +0.35 (90% CI = +0.31 to +0.39) points between treatments (see Table 2).

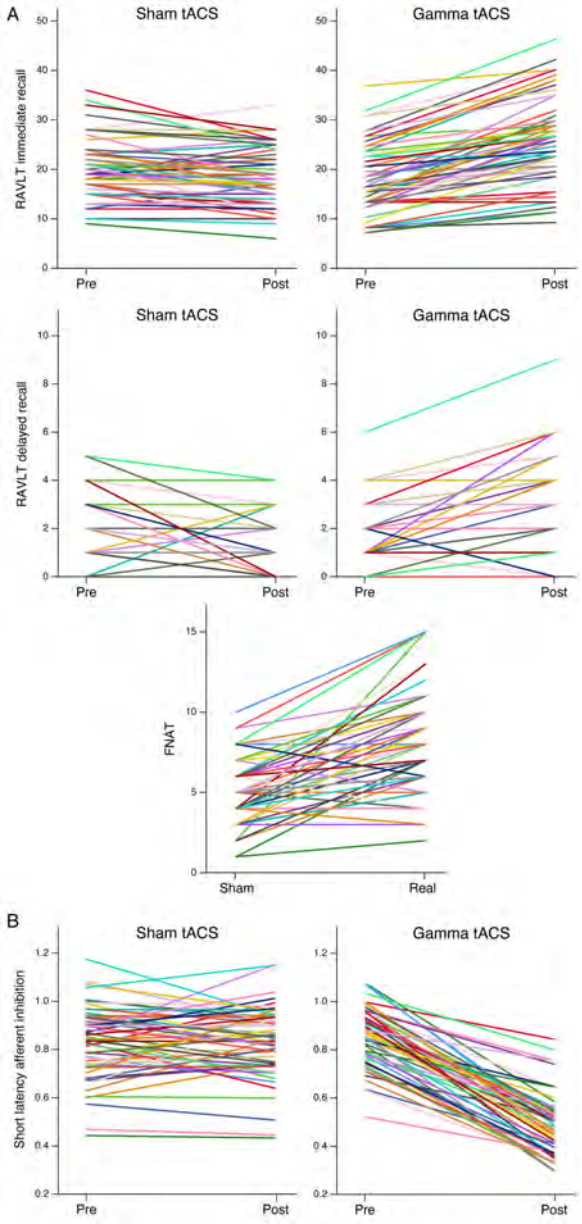

FIGURE 1: Neuropsychological and neurophysiological scores pre and post sham or γ-tACS. (A) Spaghetti plots of RAVL total recall, RAVL long delayed recall, FNAT scores. (B) Spaghetti plots of SAI measures. Legend: RAVL = Rey Auditory Verbal Learning test; FNAT = face–name associations task; tACS = transcranial alternating current stimulation; SAI = short-latency afferent inhibition. *For FNAT, results are reported during stimulation.

TABLE 2. Linear Mixed-Effects Models Output of the Cross-Over Design

Variable	RAVLT Immediate		RAVLT Delayed		FNAT		SAI	
	β ± SE	*p*-Value	β ± SE	*p*-Value	β ± SE	*p*-Value	β ± SE	*p*-Value
(Intercept)	10.2 ± 1.4	<0.001	1.45 ± 0.28	<0.001	8.20 ± 0.48	<0.001	0.13 ± 0.08	0.109
Pre-treatment test score	0.87 ± 0.07	<0.001	0.72 ± 0.09	<0.001	-	-	0.43 ± 0.09	<0.001
Treatment (real vs. sham)	−7.02 ± 0.70	<0.001	−1.61 ± 0.25	<0.001	−2.95 ± 0.42	<0.001	0.35 ± 0.02	<0.001
Block (T1 vs. T2)	−1.89 ± 0.72	0.011	0.28 ± 0.25	0.271	0.018 ± 0.42	0.965	−0.01 ± 0.02	0.890
Randomization (SR vs RS)	−1.10 ± 0.88	0.213	−0.12 ± 0.25	0.635	0.17 ± 0.563	0.767	−0.01 ± 0.02	0.691

RAVL = Rey Auditory Verbal Learning test; FNAT = face–name associations task; SAI = short-latency afferent inhibition; T1 = first session of exposure; T2 = second session of exposure after 1 week; SR = block 1 sham and block 2 real; RS = block 1 real and block 2 sham (see Supporting Information Figure 1 for details). β ± SE = regression coefficient estimate ± standard error.

SAI restoration (i.e., the difference between post γ-tACS and pre γ-tACS), corrected for donepezil intake, directly correlated with improvement of RAVL delayed recall scores after γ-tACS (i.e., the difference between post γ-tACS and pre γ-tACS scores, $r = 0.271$, $p = 0.038$) as well as with the difference of FNAT scores (i.e., the difference between the score during γ-tACS and during sham-tACS, $r = 0.307$, $p = 0.018$). No significant correlations between SAI restoration and improvement of RAVL immediate recall scores was found (see Supporting Information Figure S3).

Predictors of γ-tACS Efficacy

As an exploratory analysis, we examined the predictors of improvement after γ-tACS (i.e., the difference between post γ-tACS and pre γ-tACS scores, or γ-tACS *vs.* sham tACS).

Of the considered predictors of RAVL immediate recall improvement, *ApoE* genotype and MMSE scores were statistically significant ($p < 0.001$, see Table 3).

In particular, the greatest improvement was observed in *ApoE* ε4 non-carriers (9.6 ± 4.2 points), with a progressive loss of RAVL immediate recall improvement in heterozygous *ApoE* ε4 carriers (−3.58, 90%CI = −4.86

TABLE 3. Predictors of Memory Improvement After Real γ-tACS Stimulation

	RAVLT Immediate Improvement°			RAVLT Delayed Improvement°			FNAT Difference^		
Variable	β	90%CI	p-Value	β	90%CI	p-Value	β	90%CI	p-Value
Age at onset, year	−0.10	−0.23 to 0.02	0.17	**−0.06**	**−0.10 to − 0.01**	**0.03**	−0.04	−0.13 to 0.05	0.47
Sex, male	1.15	−0.54 to 2.84	0.27	−0.17	−0.76 to 0.42	0.63	0.83	−0.37 to 2.05	0.26
Education, years	0.14	−0.16 to 0.44	0.14	−0.02	−0.12 to 0.09	0.80	**−0.31**	**−0.5 to − 0.10**	**0.02**
Cognitive reserve	−0.01	−0.07 to 0.07	0.93	0.02	−0.01 to 0.04	0.32	0.03	−0.02 to 0.08	0.37
MMSE score	**0.57**	**0.35 to 0.79**	**<0.001**	**0.13**	**0.05 to 0.21**	**0.01**	0.10	−0.06 to 0.26	0.33
BADL score	2.32	0.02 to 4.62	0.10	**1.08**	**0.28 to 1.89**	**0.04**	−1.6	−3.57 to 0.44	0.21
NPI score	0.01	−0.12 to 0.15	0.86	0.02	−0.03 to 0.06	0.56	−0.04	−0.14 to 0.06	0.55
ApoE (ε4 carriers)	**−3.58**	**−4.86 to − 2.30**	**<0.001**	**−0.92**	**−1.37 to − 0.47**	**0.002**	**−1.22**	**−2.1 to − 0.30**	**0.04**
BDNF (M carriers)	0.44	−1.39 to 2.26	0.70	−0.33	−0.97 to 0.30	0.39	−0.76	−2.05 to 0.54	0.35

RAVL = Rey Auditory Verbal Learning test; FNAT = face–name associations task; CI = confidence intervals; MMSE = Mini-Mental State Examination; BADL = basic activities of daily living; NPI = Neuropsychiatry Inventory; *ApoE* = aaaapolipoprotein E; *BDNF* = brain derived neurotrophic factor.

°Improvement = difference between scores after γ-ACS and scores before γ-tACS; ^ difference = difference between FNAT score during γ-tACS and FNAT score during sham-tACS.β = regression coefficient estimate; 90%CI = 90% confidence interval of β. Significant *p*-values are reported in boldface.

to −2.30) and further in *ApoE* ε4/ε4 carriers as compared to *ApoE* ε4 non-carriers (−7.16, 90%CI = −9.72 to −4.60). Moreover, the milder the disease stage (as measured by MMSE), the greater the improvement in the RAVL immediate recall (see Table 3).

When predictors of RAVL delayed recall improvement were considered, comparable results were obtained. *ApoE* genotype was found statistically significant ($p = 0.001$), with a progressive loss of RAVL delayed recall improvement from *ApoE* ε4 non-carriers (1.73 ± 1.3 points) to *ApoE* ε4 heterozygous carriers (−0.92, 90% CI = −1.37 to −0.47), and to *ApoE* ε4/ε4 carriers (−1.84, 90%CI = −2.74 to −4.60). Milder disease stage, preserved functional activities of daily living score, and younger disease onset were associated to greater RAVL delayed recall improvement (all $p < 0.05$, see Table 3).

When the difference of FNAT scores between γ-tACS and sham-tACS was considered, *ApoE* genotype was statistically significant ($p = 0.034$), the greatest difference being observed in *ApoE* ε4 non-carriers (3.6 ± 2.2 points), with a progressive score reduction in *ApoE* ε4 heterozygous carriers (−1.21, 90%CI = −2.13 to −0.30), and further in *ApoE* ε4/ε4 carriers (−2.42, 90% CI = −4.26 to −0.60). In addition, a significant direct correlation between education and FNAT improvement was found ($p = 0.019$) (see Table 3).

We did not observe any significant associations between the improvement in cognitive scores after γ-tACS and sex, cognitive reserve index, NPI scores or *BDNF* genotype (all $p > 0.05$).

EEG Analysis

EEG recordings were acquired in a subset of randomly selected 10 participants (age = 74.8 ± 3.7 years, female = 50%). Compared to pre-stimulation, immediately after γ-tACS we observed a significant relative gamma power increase (20–40 Hz) on electrodes T4 and O2, a significant decrease in theta power (3–6 Hz) at electrodes F3, T3, and T4, and a significant increase in beta power (12–20 Hz) on electrodes T5, P3, T6, and O2 (all $p < 0.05$). Relative alpha frequencies were not significantly modulated after γ-tACS (see Figure 2 and Supporting Information Figure S4).

We did not observe significant modulation of any frequency ranges when comparing pre to post sham stimulation.

We observed a significant positive correlation between the increase in gamma frequencies in the parietal lobes (average P3 and P4) and the improvement at the RAVL delayed recall (i.e., the difference between post γ-tACS and pre γ-tACS scores; $r_s = 0.724$, $p = 0.018$) and FNAT scores (i.e., the difference between the score

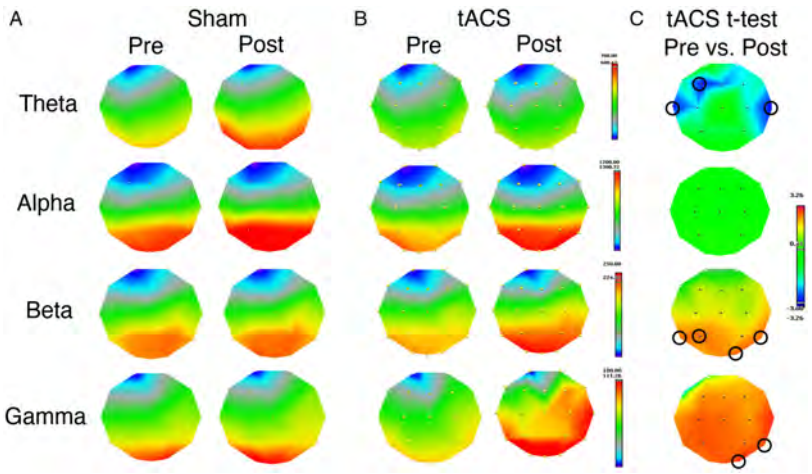

FIGURE 2: Result of the EEG frequency analysis. Frequency bands: theta (3–6 Hz), alpha (6–12 Hz), beta (12–20 Hz), gamma (20–40 Hz). (A) Power maps pre and post sham stimulation. (B) Power maps pre and post γ-tACS. (C) t-Maps of the paired t-test post vs. pre γ-tACS. Blue areas indicate a power decrease after γ-tACS, red areas a power increase. Significant effects at $p < 0.05$ were found on electrodes F3, T3, and T4 for theta (decrease after γ-tACS), on electrodes P3, T5, T6, and O2 for beta (increase after γ-tACS), and on electrodes T4 and O2 for gamma. No effect was found in the alpha band after γ-tACS and no effect was found on any bands when comparing pre vs. post sham stimulation. Legend: EEG = electroencephalography; tACS = transcranial alternating current stimulation.

during γ-tACS and during sham-tACS; $r_s = 0.815$, $p = 0.005$), but not in frontal, temporal or occipital regions (see Supporting Information Figure S5).

Computational Modelling of Electric Field Distribution

We performed individualized computation modelling of electric field distribution in a subset of thirteen participants. After precuneus segmentation, which was the hypothesized target of γ-tACS, the maximum value of the induced electric field (IEF) was 0.35 ± 0.15 V/m, whereas the mean IEF was to be 0.09 ± 0.02 V/m.

We observed a significant positive correlation between IEF electric field values and the improvement at the RAVL immediate (maximum IEF $r_s = 0.693$, $p = 0.009$; mean IEF $r_s = 0.523$, $p = 0.067$) and delayed recall (maximum IEF $r_s = 0.712$, $p = 0.006$; mean IEF $r_s = 0.697$, $p = 0.008$).

Discussion

The first goal of any treatment in AD is to improve memory functions, hopefully reverting the ongoing pathological process. Brain oscillations, which arise from synchronized interactions between neuronal populations, are essential for cognitive performances, and gamma oscillations have been shown to be associated with long-term memory-related synaptic changes in the hippocampus.[32, 33] Accordingly, AD patients present dysregulation of gamma activity, which represents an early event and might trigger clinical onset.[34–36]

In patients with mild dementia due to AD, a small number of recent studies has suggested the potential therapeutic benefits of γ-tACS,[15, 37–39] able to modulate brain activity and entrain gamma rhythms by low-amplitude alternating sinusoidal currents.[40, 41]

The present study confirms and extends previous findings suggesting a potential beneficial effect of precuneus γ-tACS on memory in AD patients. Notably, we found a significant correlation between enhancement of episodic memory after γ-tACS and the betterment of indirect measures of cholinergic neurotransmission, consistent with the link between acetylcholine levels and gamma oscillations in AD.[42–44]

The aforementioned cognitive benefits were corroborated by electrophysiological changes observed after γ-tACS stimulation in patients with AD, resulting in entrainment of gamma frequency, but also in increased beta power activity on the posterior brain regions and decreased theta power activity on the anterior brain regions. The improvement in long-term memory correlated with the increase of gamma activity over posterior regions, suggesting a site-specific effect of γ-tACS, involving structures as the posterior parietal cortices and the precuneus. Moreover, computational modelling based on individual patients' MRIs showed a positive correlation between the current that effectively reached the precuneus and the improvement after γ-tACS at the RAVL immediate and delayed recall.

It is difficult to compare the present study to recently published investigations using γ-tACS in AD,[15, 39] as we targeted the precuneus because it is one of the first regions to be affected in AD and deeply involved in associative and episodic memory.[45]

Moreover, compared to most of the studies in the literature, we employed tACS rather than other non-invasive stimulation methods.[46] Indeed, as compared to transcranial direct current stimulation (tDCS), tACS is able to enhance synchronization of cortical oscillations beyond restoring brain plasticity,[47] and as compared to repetitive transcranial magnetic stimulation (rTMS), tACS may be easily delivered in at-home settings.[48, 49] In this view, a recent pilot study carried out on two AD patients demonstrated the safety of home-based, remotely-monitored and caregiver-administered tACS intervention.[15]

We selected the precuneus as the target area to be stimulated, because it is one of the first regions to be affected in prodromal AD and the hub of the default mode network (DMN), functionally connected to mesial temporal regions, while being easily accessible by transcranial stimulation. Alterations of the DMN have been identified as responsible of memory impairment[50–52] and the precuneus has been described as a key node for memory functioning.[53, 54] Recently, high-frequency rTMS over the precuneus has demonstrated an improvement in episodic memory associated to brain connectivity changes in AD patients, suggesting that precuneus stimulation might represent a useful technique in order to enhance episodic memory.[55] The improvement observed at the FNAT, which has been shown to depend primarily on hippocampal structures,[56] could be explained by the entrainment of large-scale cortical network activity by network resonance, functionally connected to the precuneus *via* the DMN[57] consistent with previous studies showing that parietal cortical stimulation modulates neural activity in a hippocampal-cortical network that supports episodic memory processing.[58, 59]

Interestingly, we also identified the predictors of tACS efficacy. Our study clearly indicated that an increased response pattern was influenced by *ApoE* genotype, with greater cognitive improvement in *ApoE* ε4 non-carriers, and with a progressive loss of stimulation efficacy in subjects with at least one *ApoE* ε4 allele, and even more in subjects with two *ApoE* ε4 alleles. This is in agreement

with previous studies emphasizing that interventions designed to modify cholinergic transmission[60] or to alter amyloid load in AD may interact with *ApoE* genotype, resulting in differential efficacy and outcome.[61] This observation may be an important factor to consider in future trial design. We did not observe a significant effect of *BDNF* genotype nor for cognitive reserve on clinical improvement after γ-tACS. Furthermore, as reported in previous AD pharmacological treatments,[62] we found that milder disease stage was associated with greater improvement of episodic memory performances after stimulation.

The observed effects on the primary outcomes, i.e., the improvement in RAVL immediate and delayed recall and FNAT scores, after γ-tACS, although small, were still large enough to meet the criteria for minimal clinically important difference (MCID), that is defined as the smallest change in a treatment outcome that an individual patient would identify as important and which would indicate a change in the patient's management.[63] Moreover, we demonstrated that these effects were site specific (i.e., γ-tACS over the dorsolateral prefrontal cortex did not improve memory performances) and specifically restored memory functions (i.e., γ-tACS over the precuneus did not improve other cognitive domains).

We acknowledge that this study entails some limits. First, we evaluated the effects of a single session of γ-tACS over the precuneus, but long-term effects need to be assessed in multisession trials. Second, a larger, multicenter sample of subjects may further strengthen the results and account for possible confounders. However, we applied a crossover trial, which is statistically efficient and requires fewe subjects than non-crossover designs; further, the influence of confounding covariates is reduced because each subject serves as his or her own control.[64] Third, the change in RAVL delayed recall scores, although statistically significant, was minimal, particularly considering that patients at this stage have virtually absent delayed recall on the RAVL. Fourth, we did not assess long-term effects after γ-tACS, which are probably unrealistic to see after a single 60-minute stimulation, and should be ideally evaluated after multiple repeated sessions of γ-tACS. Finally, learning effects should be considered, even though we administered different sets of memory tests during the experimental neuropsychological assessment.

In conclusion, γ-tACS over the precuneus can safely and efficiently induce entrainment of neural oscillations in patients with AD, improving memory functions and ameliorating cholinergic deficits. The refinement of predictors of outcome may best identify patients who may benefit most from γ-tACS stimulation.

These findings suggest that γ-tACS stimulation over the precuneus may represent a novel therapeutic approach in AD. Future studies with multisession γ-tACS and with at-home setting design are warranted.

Acknowledgments

The authors thank all patients for their participation in this research, and Ilenia Libri and Jasmine Rivolta for the great support in neurophysiological and clinical evaluations. The present work was supported by the Airalzh-AGYR2020 grant issued to AB and by the Italian Ministry of Health (Ricerca Corrente), issued to MC. Open Access Funding provided by Universita degli Studi di Brescia within the CRUI-CARE Agreement.

Author Contributions

A.B. and B.B. contributed to the conception and design of the study; A.B., V.C., M.G., L.B., C.M.M., A.D., C.T., S.G., M.S.C., M.B., E.P., Y.G., M.C., M.P., F.P., M.S., S.A., E.S., A.P., A.P.L., and B.B. contributed to the acquisition and analysis of data; A.B., L.B., and B.B. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

A.B. and B.B. have pending patent on the use of non-invasive brain stimulation to increase memory functions in patients with Alzheimer Disease.

References

1. Cummings J, Lee G, Ritter A, et al. Alzheimer's disease drug development pipeline: 2020. Alzheimer's Dement. Transl Res Clin Interv 2020;6:e12050.
2. Hampel H, Mesulam MM, Cuello AC, et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain 2018;141:1917–1933.
3. Sevigny J, Chiao P, Bussière T, et al. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease [internet]. Nature 2016; 537:50–56. Available from: https://doi.org/10.1038/nature19323.
4. FDA Grants Accelerated Approval for Alzheimer's Drug [Internet]. 2021 [cited 2021 Jul 6] Available from: https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-alzheimers-drug
5. Koenig T, Prichep L, Dierks T, et al. Decreased EEG synchronization in Alzheimer's disease and mild cognitive impairment [internet]. Neurobiol Aging 2005;26:165–171.[cited 2019 Oct 9] Available from:. https://www.sciencedirect.com/science/article/pii/S0197458004001538?via%3Dihub.
6. Babiloni C, Lizio R, Marzano N, et al. Brain neural synchronization and functional coupling in Alzheimer's disease as revealed by resting state EEG rhythms [internet]. Int J Psychophysiol 2016;103:88–102. Available from. https://doi.org/10.1016/j.ijpsycho.2015.02.008.
7. Iaccarino HF, Singer AC, Martorell AJ, et al. Gamma frequency entrainment attenuates amyloid load and modifies microglia [internet]. Nature 2016;540:230–235. Available from. https://doi.org/10.1038/nature20587.

8. Martorell AJ, Paulson AL, Suk H, et al. Multi-sensory gamma stimulation ameliorates Alzheimer's-associated pathology and improves cognition. [internet]. Cell 2019;177:256–271.e22. Available from. https://doi.org/10.1016/j.cell.2019.02.014.

9. Adaikkan C, Middleton SJ, Marco A, et al. Gamma entrainment binds higher-order brain regions and offers neuroprotection [internet]. Neuron 2019;102:1–15. Available from. https://linkinghub.elsevier.com/retrieve/pii/S0896627319303460.

10. Herrmann CS, Murray MM, Ionta S, et al. Shaping intrinsic neural oscillations with periodic stimulation. J Neurosci 2016;36:5328–5337.

11. Antal A, Paulus W. Transcranial alternating current stimulation (tACS). Front Hum Neurosci 2013;7:1–4.

12. Fröhlich F, Sellers KK, Cordle AL. Targeting the neurophysiology of cognitive systems with transcranial alternating current stimulation. Expert Rev Neurother 2014;15:145–167.

13. Vosskuhl J, Strüber D, Herrmann CS. Non-invasive brain stimulation: a paradigm shift in understanding brain oscillations. Front Hum Neurosci 2018;12:1–19.

14. Herrmann CS, Rach S, Neuling T, Strüber D. Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. [Internet]. Front Hum Neurosci 2013;7:279. Available from. http://www.ncbi.nlm.nih.gov/pubmed/23785325.

15. Bréchet L, Yu W, Biagi MC, et al. Patient-tailored, home-based non-invasive brain stimulation for memory deficits in dementia due to Alzheimer's disease. Front Neurol 2021;12:1–12.

16. Benussi A, Cantoni V, Cotelli MS, et al. Exposure to gamma tACS in Alzheimer's disease: a randomized, double-blind, sham-controlled, crossover, pilot study [internet]. Brain Stimul 2021;14:531-540. https://linkinghub.elsevier.com/retrieve/pii/S1935861X21000589.

17. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. [internet]. Science 1993;261:921–923. Available from. http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=8346443&retmode=ref&cmd=prlinks.

18. Riddle J, McPherson T, Atkins AK, et al. Brain-derived neurotrophic factor (BDNF) polymorphism may influence the efficacy of tACS to modulate neural oscillations [internet]. Brain Stimul 2020;13:998–999. https://doi.org/10.1016/j.brs.2020.04.012.

19. Guerra A, Asci F, Zampogna A, et al. Gamma-transcranial alternating current stimulation and theta-burst stimulation: inter-subject variability and the role of BDNF [internet]. Clin Neurophysiol 2020;131:2691–2699. https://doi.org/10.1016/j.clinph.2020.08.017.

20. Jack CR, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease [Internet]. Alzheimers Dement 2018;14:535–562.Available from. https://linkinghub.elsevier.com/retrieve/pii/S1552526018300724.

21. Benussi A, Dell'Era V, Cantoni V, et al. TMS for staging and predicting functional decline in frontotemporal dementia. [Internet]. Brain Stimul 2020;13:386–392. Available from:. http://www.ncbi.nlm.nih.gov/pubmed/31787557.

22. Nucci M, Mapelli D, Mondini S. Cognitive reserve index questionnaire (CRIq): a new instrument for measuring cognitive reserve. Aging Clin Exp Res 2012;24:218–226.

23. Padovani A, Benussi A, Cantoni V, et al. Diagnosis of mild cognitive impairment due to Alzheimer's disease with transcranial magnetic stimulation. [Internet] J Alzheimers Dis 2018;65:221–230. Available from. http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=30010131&retmode=ref&cmd=prlinks.

24. Kasten FH, Dowsett J, Herrmann CS. Sustained aftereffect of α-tACS lasts up to 70 min after stimulation. Front Hum Neurosci 2016;10:1–9.

25. Rey A. L'Examen Clinique en Psychologie [clinical examination in psychology]. 1964.

26. Rentz DM, Amariglio RE, Becker JA, et al. Face-name associative memory performance is related to amyloid burden in normal elderly [internet]. Neuropsychologia 2011;49:2776–2783. Available from. https://doi.org/10.1016/j.neuropsychologia.2011.06.006.

27. Jung TP, Makeig S, Humphries C, et al. Removing electroencephalographic artifacts by blind source separation. Psychophysiology 2000;37:163–178.

28. Benussi A, Cosseddu M, Filareto I, et al. Impaired long-term potentiation-like cortical plasticity in presymptomatic genetic frontotemporal dementia. [internet]. Ann Neurol 2016;80:472–476. https://doi.org/10.1002/ana.24731.

29. Benussi A, Grassi M, Palluzzi F, et al. Classification accuracy of transcranial magnetic stimulation for the diagnosis of neurodegenerative dementias. [internet]. Ann Neurol 2020;87:394–404. Available from. http://www.ncbi.nlm.nih.gov/pubmed/31925823.

30. Tokimura H, Di Lazzaro V, Tokimura Y, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. [internet]. J Physiol 2000;523:503–513. https://doi.org/10.1111/j.1469-7793.2000.t01-1-00503.x.

31. Mehta AR, Pogosyan A, Brown P, Brittain JS. Montage matters: the influence of transcranial alternating current stimulation on human physiological tremor [internet]. Brain Stimul 2015;8:260–268. https://doi.org/10.1016/j.brs.2014.11.003.

32. Fernández-Ruiz A, Oliva A, Soula M, et al. Gamma rhythm communication between entorhinal cortex and dentate gyrus neuronal assemblies. Science 2021;372:eabf3119.

33. Yamamoto J, Suh J, Takeuchi D, Tonegawa S. Successful execution of working memory linked to synchronized high-frequency gamma oscillations [internet]. Cell 2014;157:845–857. https://doi.org/10.1016/j.cell.2014.04.009.

34. Scott L, Feng J, Kiss T, et al. Age-dependent disruption in hippocampal theta oscillation in amyloid-β overproducing transgenic mice [internet]. Neurobiol Aging 2012;33:1481.e13–1481.e23. https://doi.org/10.1016/j.neurobiolaging.2011.12.010.

35. Başar E, Emek-Savaş DD, Güntekin B, Yener GG. Delay of cognitive gamma responses in Alzheimer's disease. NeuroImage Clin 2016;11:106–115.

36. Grunwald M, Busse F, Hensel A, et al. Correlation between cortical θ activity and hippocampal volumes in health, mild cognitive impairment, and mild dementia. J Clin Neurophysiol 2001;18:178–184.

37. Naro A, Corallo F, De Salvo S, et al. Promising role of Neuromodulation in predicting the progression of mild cognitive impairment to dementia. J Alzheimers Dis 2016;53:1375–1388.

38. Xing Y, Wei P, Wang C, et al. TRanscranial AlterNating current stimulation FOR patients with mild Alzheimer's disease (TRANSFORM-AD study): protocol for a randomized controlled clinical trial. Alzheimer's Dement Transl Res Clin Interv 2020;6:1–8.

39. Kim J, Kim H, Jeong H, et al. tACS as a promising therapeutic option for improving cognitive function in mild cognitive impairment: a direct comparison between tACS and tDCS [internet]. J Psychiatr Res 2021;141:248–256. https://doi.org/10.1016/j.jpsychires.2021.07.012.

40. Johnson L, Alekseichuk I, Krieg J, et al. Dose-dependent effects of transcranial alternating current stimulation on spike timing in awake nonhuman primates [Internet]. Sci.Adv 2020;6:eaaz2747. Available from. https://doi.org/10.1126/sciadv.aaz2747.

41. Beliaeva V, Polania R. Can low-intensity tACS genuinely entrain neural activity in vivo? Brain Stimul 2020;13:1796–1799.

42. Amat-Foraster M, Leiser SC, Herrik KF, et al. The 5-HT6 receptor antagonist idalopirdine potentiates the effects of donepezil on gamma oscillations in the frontal cortex of anesthetized and awake rats without affecting sleep-wake architecture [internet]. Neuropharmacology 2017;113:45–59. https://doi.org/10.1016/j.neuropharm.2016.09.017.

43. Spencer JP, Middleton LJ, Davies CH. Investigation into the efficacy of the acetylcholinesterase inhibitor, donepezil, and novel

procognitive agents to induce gamma oscillations in rat hippocampal slices [internet]. Neuropharmacology 2010;59:437–443. https://doi.org/10.1016/j.neuropharm.2010.06.005.

44. Babiloni C, Del Percio C, Bordet R, et al. Effects of acetylcholinesterase inhibitors and memantine on resting-state electroencephalographic rhythms in Alzheimer's disease patients [internet]. Clin Neurophysiol 2013;124:837–850. https://doi.org/10.1016/j.clinph.2012.09.017.

45. Cavanna AE, Trimble MR. The precuneus: a review of its functional anatomy and behavioural correlates. Brain 2006;129:564–583.

46. Woods AJ, Antal A, Bikson M, et al. A technical guide to tDCS, and related non-invasive brain stimulation tools [internet]. Clin Neurophysiol 2016;127:1031–1048. Available from. http://linkinghub.elsevier.com/retrieve/pii/S1388245715010883.

47. Inukai Y, Saito K, Sasaki R, et al. Comparison of three non-invasive transcranial electrical stimulation methods for increasing cortical excitability. [internet]. Front Hum Neurosci 2016;10:668. Available from. http://www.journal.frontiersin.org/article/10.3389/fnhum.2016.00668/full.

48. Sivaramakrishnan A, Datta A, Bikson M, Madhavan S. Remotely supervised transcranial direct current stimulation: a feasibility study for amyotrophic lateral sclerosis. NeuroRehabilitation 2019;45:369–378.

49. Charvet LE, Shaw MT, Bikson M, et al. Supervised transcranial direct current stimulation (tDCS) at home: a guide for clinical research and practice [internet]. Brain Stimul 2020;13:686–693. Available from. https://doi.org/10.1016/j.brs.2020.02.011.

50. Huo L, Li R, Wang P, et al. The default mode network supports episodic memory in cognitively unimpaired elderly individuals: different contributions to immediate recall and delayed recall. Front Aging Neurosci 2018;10:1–10.

51. Whitfield-Gabrieli S, Ford JM. Default mode network activity and connectivity in psychopathology. Annu Rev Clin Psychol 2012;8:49–76.

52. Small GW, Ercoli LM, Silverman DHS, et al. Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. Proc Natl Acad Sci U S A 2000;97:6037–6042.

53. Lundstrom BN, Petersson KM, Andersson J, et al. Isolating the retrieval of imagined pictures during episodic memory: activation of the left precuneus and left prefrontal cortex. Neuroimage 2003;20:1934–1943.

54. Wagner AD, Shannon BJ, Kahn I, Buckner RL. Parietal lobe contributions to episodic memory retrieval. Trends Cogn Sci 2005;9:445–453.

55. Koch G, Bonnì S, Pellicciari MC, et al. Transcranial magnetic stimulation of the precuneus enhances memory and neural activity in prodromal Alzheimer's disease [internet]. Neuroimage 2018;169:302–311. https://doi.org/10.1016/j.neuroimage.2017.12.048.

56. Kirwan CB, Stark CEL. Medial temporal lobe activation during encoding and retrieval of novel face-name pairs. Hippocampus 2004;14:919–930.

57. Ali MM, Sellers KK, Fröhlich F. Transcranial alternating current stimulation modulates large-scale cortical network activity by network resonance. J Neurosci 2013;33:11262–11275.

58. Meng A, Kaiser M, de Graaf TA, et al. Transcranial alternating current stimulation at theta frequency to left parietal cortex impairs associative, but not perceptual, memory encoding [internet]. Neurobiol. Learn. Mem 2021;182:107444. https://doi.org/10.1016/j.nlm.2021.107444.

59. Freedberg M, Reeves JA, Toader AC, et al. Persistent enhancement of hippocampal network connectivity by parietal rTMS is reproducible. eNeuro 2019;6:1–13.

60. Cacabelos R. Pharmacogenetic considerations when prescribing cholinesterase inhibitors for the treatment of Alzheimer's disease [internet]. Expert Opin Drug Metab Toxicol 2020;16:673–701. https://doi.org/10.1080/17425255.2020.1779700.

61. Verghese PB, Castellano JM, Holtzman DM. Apolipoprotein E in Alzheimer's disease and other neurological disorders [internet]. Lancet Neurol 2011;10:241–252. https://doi.org/10.1016/S1474-4422(10)70325-2.

62. Andrieu S, Coley N, Lovestone S, et al. Prevention of sporadic Alzheimer's disease: lessons learned from clinical trials and future directions [internet]. Lancet Neurol 2015;14:926–944. Available from. http://linkinghub.elsevier.com/retrieve/pii/S1474442215001532.

63. Wright A, Hannon J, Hegedus EJ, Kavchak AE. Clinimetrics corner: a closer look at the minimal clinically important difference (MCID). J Man Manip Ther 2012;20:160–166.

64. Honnorat J, Antoine J-C, Saiz A, et al. Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies: study of 14 patients. [internet]. Arch Neurol 2001;58:225–230. Available from. http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=11176960&retmode=ref&cmd=prlinks.

Sprugnoli *et al.*
Alzheimer's Research & Therapy (2021) 13:203
https://doi.org/10.1186/s13195-021-00922-4

Alzheimer's Research & Therapy

RESEARCH Open Access

Impact of multisession 40Hz tACS on hippocampal perfusion in patients with Alzheimer's disease

Giulia Sprugnoli[1,2], Fanny Munsch[3], Davide Cappon[1], Rachel Paciorek[1], Joanna Macone[1], Ann Connor[1], Georges El Fakhri[4], Ricardo Salvador[5], Giulio Ruffini[5], Kevin Donohoe[4], Mouhsin M. Shafi[1], Daniel Press[1], David C. Alsop[3], Alvaro Pascual Leone[6,7,8] and Emiliano Santarnecchi[9*]

Abstract

Background: Alzheimer's disease (AD) is associated with alterations in cortical perfusion that correlate with cognitive impairment. Recently, neural activity in the gamma band has been identified as a driver of arteriolar vasomotion while, on the other hand, gamma activity induction on preclinical models of AD has been shown to promote protein clearance and cognitive protection.

Methods: In two open-label studies, we assessed the possibility to modulate cerebral perfusion in 15 mild to moderate AD participants via 40Hz (gamma) transcranial alternating current stimulation (tACS) administered 1 h daily for 2 or 4 weeks, primarily targeting the temporal lobe. Perfusion-sensitive MRI scans were acquired at baseline and right after the intervention, along with electrophysiological recording and cognitive assessments.

Results: No serious adverse effects were reported by any of the participants. Arterial spin labeling MRI revealed a significant increase in blood perfusion in the bilateral temporal lobes after the tACS treatment. Moreover, perfusion changes displayed a positive correlation with changes in episodic memory and spectral power changes in the gamma band.

Conclusions: Results suggest 40Hz tACS should be further investigated in larger placebo-controlled trials as a safe, non-invasive countermeasure to increase fast brain oscillatory activity and increase perfusion in critical brain areas in AD patients.

Trial registration: Studies were registered separately on ClinicalTrials.gov (NCT03290326, registered on September 21, 2017; NCT03412604, registered on January 26, 2018).

Keywords: Dementia, Cerebral blood flow, Neuromodulation, Neurostimulation, tES, Gamma band, Gamma activity, Hippocampus, EEG, CBF

Background

Alzheimer's disease (AD) is the most common cause of dementia, and its prevalence continues to increase [1]. Despite this enormous disease burden and intensive scientific research, therapeutic options are limited. While there are pharmacologic interventions that transiently stabilize cognitive function, no disease-modifying therapy is available. Even promising pharmacological

*Correspondence: esantarnecchi@mgh.harvard.edu
[9] Precision Neuroscience & Neuromodulation Program, Gordon Center for Medical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
Full list of author information is available at the end of the article

interventions (e.g., aducanumab, an anti-amyloid compound) do not appear to stop cognitive decline [2]. In an effort to develop effective treatments, research has then focused on deepening our understanding of the pathophysiology of AD. Positron emission tomography (PET) as well as single-photon emission computed tomography (SPECT) imaging revealed marked hypometabolism and perfusion deficits in AD patients with respect to healthy controls [3]. More recently, a perfusion-sensitive MRI imaging sequence, arterial spin labeling (ASL), has been developed to study brain perfusion without the need for contrasting agents [4]. ASL has helped reveal a significant reduction of brain perfusion (cerebral blood flow - CBF) in the temporal, parietal, and posterior cingulate cortices in AD patients with respect to healthy subjects [3, 5], even though a pathological blood flow increase in preclinical stages of the disease has also been described [6, 7]. Moreover, CBF reduction has been correlated with language impairment in AD patients [8], and its reduction is paralleled by disease progression starting from the prodromal stage [9]. Also, hypoperfusion may predict conversion to AD in mild cognitive impairment (MCI) patients [10], making the quest for approaches to modulate (i.e., increase) perfusion a priority.

A recent preclinical animal study has shown that gamma (γ) band oscillatory brain activity is directly responsible for arteriolar vasodilatation and the consequent increase in blood oxygenation [11]. Gamma activity usually refers to cortical oscillations in the 30–80Hz frequency band, primarily generated by the interaction between inhibitory interneurons such as parvalbumin (PV)+ interneurons, and pyramidal cells [12]. The investigators found that optogenetic manipulations of γ-band electrical power entrain the vasomotor oscillations of corresponding cortical and penetrating arterioles in a unidirectional way, i.e., independently from baseline CBF [11]. In turn, fluctuations in arteriolar diameter coherently drive fluctuations in blood oxygenation [11]. Electrical activity drives the arteriolar vasomotion with a lag of 2 s, which in turn leads to changes in functional MRI signal (detecting increases of oxygenation in the venular component; BOLD signal) one second later [11]. The significance of this electro-arteriolar coupling has been related to the role of vasomotion in the removal of waste and toxic proteins via the paravascular space, i.e., the so-called glymphatic system, that seems to be impaired in AD mouse models and AD patients [13, 14].

Pivotal preclinical studies have also shown that exogenously-induced increase of gamma oscillations (specifically at 40Hz) promotes microglial activation and cause subsequent reduction of Aβ and p-tau depositions in a mouse model of AD [15]. Decreased γ activity in AD mouse models is linked to PV+ inhibitory interneuron pathology that interferes with fast inhibitory loops in cortical circuits and is associated with a hyperactivation of pyramidal cells leading to global network dysfunction [16]. Remarkably, induction of gamma activity in presymptomatic AD mice—and thus a restoration of the physiological activity of PV interneurons—prevents subsequent neurodegeneration and behavioral deficits [17]. As seen in preclinical models [18], a consistent finding in patients with AD is a relative attenuation and dysregulation of gamma activity [19], therefore gamma induction may represent a novel and powerful therapeutic approach [17].

Recently, a neuromodulation technique that delivers alternating current stimulation—transcranial alternating current stimulation (tACS)—has received attention for the possibility of translating the aforementioned animal evidence to humans via noninvasive induction of gamma activity [20]. tACS applies low-amplitude alternating (sinusoidal) current to enhance specific oscillations by entraining neurons under specific cortical rhythms, depending on the applied stimulation frequency (e.g., 40Hz) [21]. Non-human animal work has demonstrated that tACS entrains neurons in widespread cortical areas [22], with recent non-human primates experiments revealing dose-dependent neural entrainment and increased burstiness as the fundamental response to tACS [23, 24]. Simulations, supported by empirical evidence using electroencephalography (EEG), demonstrated that tACS modulates brain oscillatory activity via network resonance, suggesting that a weak stimulation at a resonant frequency could cause large-scale modulation of network activity [25], and amplify endogenous network oscillations in a frequency-specific manner [18]. Consequently, tACS has been found able to modulate brain oscillation and related behavior in healthy subjects and patients [26–28], with an enhancement of gamma oscillations via tACS leading to transient improvement in motor, working memory, and abstract reasoning tasks on healthy controls [29–31], and effects often lasting beyond the tACS application period [30, 32, 33].

Given the evidence of impaired gamma activity in AD patients, and the potential of restoring brain perfusion via gamma entrainment, in the present pilot studies, we aimed to translate to humans the aforementioned preclinical findings on 40Hz gamma stimulation in animal models of AD by means of tACS applied to a sample of 15 mild to moderate AD patients. We hypothesize that a multiday course of tACS would lead to an increase in CBF in regions targeted by tACS, with a stronger effect for participants receiving longer tACS treatments. Additionally, we hypothesized that CBF changes would show some spatial specificity in relation to the different tACS electrode montages used in the studies, and potentially

covariation with changes in the spectral power of gamma as measured via EEG as well as episodic memory scores indexing temporal lobe/hippocampal function.

Methods

Participants and experimental design

Fifteen participants with mild to moderate dementia due to AD were enrolled in total (mean age 72 years, male = 9; Mini-Mental State Examination - MMSE = 23.53, SD = 3.35). Participants were enrolled in two separate open-label clinical trials exploring the impact of different tACS doses (i.e., number of stimulation sessions) and targeting approaches (i.e., positioning of tACS electrodes on the scalp and resulting induced electrical field in the brain). Participants received 1 h of daily tACS for 2 or 4 weeks in hospital settings (Monday to Friday), with baseline (pre-tACS) and follow-up (post-tACS) assessments composed of cognitive and memory testing, EEG, and perfusion MRI (ASL) data. Participants underwent additional assessments pre/post tACS not reported in the present manuscript and beyond the scope of the present study, e.g., PET imaging for Aβ and p-tau, transcranial magnetic stimulation (TMS) measures, combined TMS-EEG recording, voice biomarkers recording, blood biomarkers.

Depending on the tACS paradigms, participants can be subdivided into three subgroups: (i) subjects receiving 2 weeks (10 sessions = 10h) of unilateral temporo-frontal tACS (Group 1; *n*=5); (ii) subjects receiving 2 weeks (10 sessions = 10h) of bitemporal tACS (Group 2; *n*=5); (iii) subjects receiving 4 weeks (20 sessions = 20h) of bitemporal tACS (Group 3; *n*=5) (Fig. 1). Common site of stimulation across montages was represented by the right temporal lobe (Fig. 1). Within a 1-week period before and after the tACS intervention, participants underwent a cognitive assessment battery, 64 channels scalp EEG, and MRI assessments. All participants gave written informed consent prior to participating in the studies, registered separately on ClinicalTrials.gov (NCT03412604, NCT03290326; PI Santarnecchi).

The first pilot trial NCT03290326 was designed to assess safety and feasibility of performing a two-weeks stimulation tACS treatment in patients with AD. Among the first 10 patients enrolled (Groups 1 and 2 in the manuscript), Group 1 underwent a personalized right-sided stimulation centered on the temporo-frontal lobes. The rationale was to personalize stimulation on the basis of individual Aβ accumulation maps based on Florbetapir PET imaging. In Group 1 participants, regions with higher Aβ load on the right temporal and frontal lobes were targeted, with stronger tACS intensity over the temporal lobe given the higher load of Aβ compared to the frontal lobe (Fig. 1). This approach led to Group 1 subjects receiving stimulation in the right temporo-frontal lobes; however, the electrodes of maximal injected current were slightly different across participant on the basis of the distribution of their amyloid load (i.e., maximal current density on EEG electrode positions F4 and T8 in patient X, F2 and T8 in patient Y). The unilateral personalized stimulation was conceived as a method to (i) test the spatial specificity of tACS stimulation on the individual target regions in the AD brain, (ii) improve the localization and/or recognition of ictal/EEG changes during and/or after the treatment (i.e., more probable in the stimulated right hemisphere). Given that in the first 5 patients (Group 1) a very good spatial localization was achieved and no epileptiform alterations were detected at the electrophysiological as well as clinical assessments, the investigators decided to stimulate Group 2 bilaterally, specifically over the temporal lobes.

Bitemporal tACS was conceived as a way to induce more focal stimulation over the temporal lobes, given the typical Aβ and tau protein distribution in the AD brain [34]. Additionally, tau protein, particularly expressed in the temporal lobe, is also significantly correlated with cognitive decline in AD patients, compared to a weak to null association for Aβ. Therefore, more emphasis on the temporal lobe would guarantee a higher chance of potentially modulating tau accumulation in the future, while also still targeting hypoperfusion present in the same area in AD patients. Finally, in the NCT03412604 trial corresponding to Group 3, bitemporal stimulation was conducted for 4 weeks to enhance the probability of inducing perfusion and protein changes, while at the same time testing the safety and feasibility of a 4-weeks stimulation protocol. The research proposal and associated methodologies were approved by the local ethics committee (Beth Israel Deaconess Medical Center IRB) in accordance with the principles of the Declaration of Helsinki.

Transcranial alternating stimulation (tACS)

tACS was delivered via a battery-driven current stimulator (Starstim SS32, Neuroelectrics, Barcelona, Cambridge) through surface circular Ø 20mm PISTIM electrodes (Neuroelectrics, Barcelona, Spain) with an Ag/AgCl core and a gel/skin contact area of 3.14 cm^2. The electrodes were placed into holes of a neoprene cap corresponding to the international 10/20 EEG system. Gel (Signa, PARKER LABORATORIES, INC.) was applied to optimize signal conductivity and lower impedance. Electrode impedance was checked before starting each tACS session to assure safety and maximal efficacy of stimulation as well as to ensure familiarization of participants with the tACS-induced scalp sensations (e.g., tingling). For all sessions, 32 electrodes were placed on the scalp to

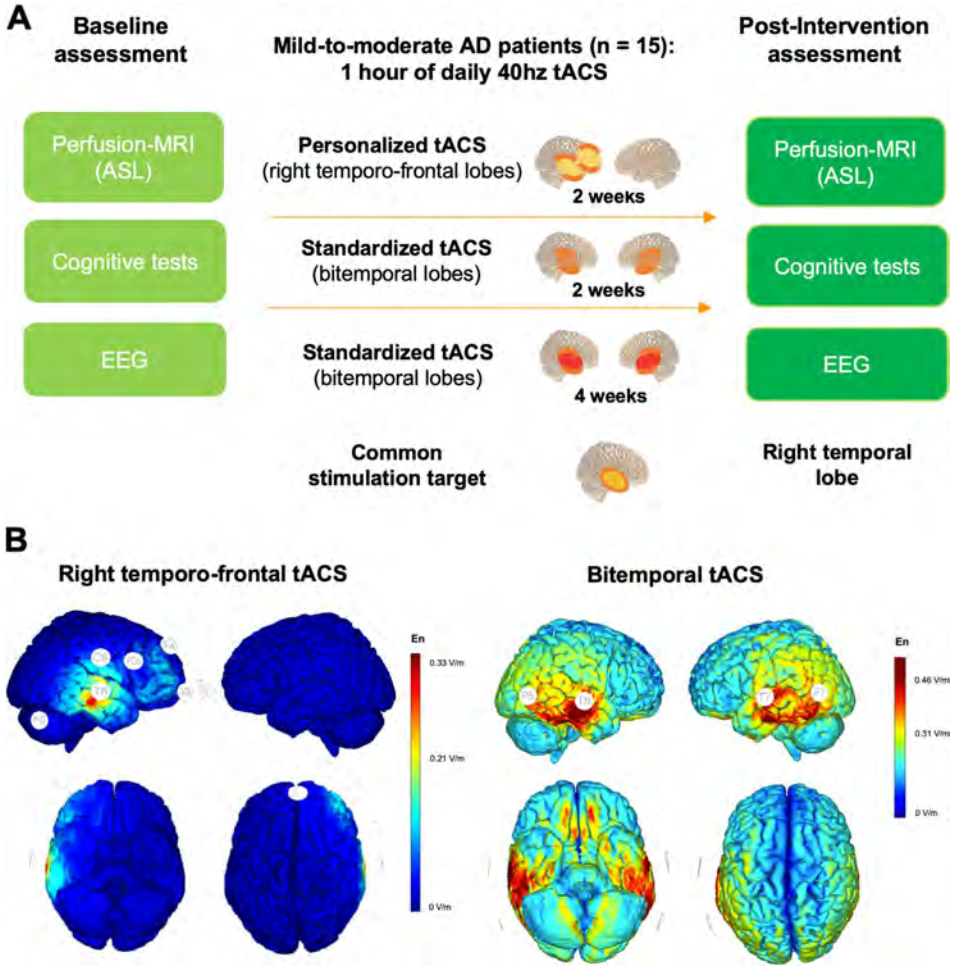

Fig. 1 Experimental protocol. **A** Study design and relevant pre-post tACS measures. Fifteen participants with mild to moderate dementia due to AD were enrolled in total (mean age 72 years, male = 9; MMSE = 23.53, SD = 3.35). Participants received 1 h of daily tACS for 2 or 4 weeks in hospital settings (Monday to Friday), with baseline (pre-tACS) and follow-up (post-tACS) assessments composed of cognitive and memory testing, EEG, and perfusion MRI (ASL) data. Participants underwent additional assessments pre/post tACS not reported in the present manuscript and beyond the scope of the present study, e.g. PET imaging for Aβ and p-tau, transcranial magnetic stimulation (TMS) measures, combined TMS-EEG recording, voice biomarkers recording, blood biomarkers. tACS was conducted targeting the normal component of the electric field either to the bilateral temporal lobes (bitemporal tACS hereafter) or unilateral (right) temporal and frontal lobes (temporo-frontal tACS hereafter), thus always impacting the right temporal lobe across all participants (corresponding to T8 on the 10/20 EEG system). Therefore, participants can be subdivided into three subgroups: (i) subjects receiving 2 weeks (10 sessions = 10h) of unilateral temporo-frontal tACS (Group 1; *n*=5); (ii) subjects receiving 2 weeks (10 sessions = 10h) of bitemporal tACS (Group 2; *n*=5); (iii) subjects receiving 4 weeks (20 sessions = 20h) of bitemporal tACS (Group 3; *n*=5). Common site of stimulation across montages was represented by the right temporal lobe. **B** On the left, normal electrical field (En-field) for representative subject receiving unilateral temporo-frontal tACS (Group 1), on the right En-field for participants receiving bilateral temporal lobe stimulation (Groups 2 and 3)

record EEG before and after each tACS session, although only a subset of the electrodes was used to deliver tACS [35]. tACS at a stimulation frequency of 40Hz was applied for 1 h with a maximum intensity of 2mA on each electrode and 4mA total across all electrodes, preceded by a 30-s ramp up period and followed by a 30-s ramp down period, while research and clinical personnel carefully monitored for side effects for the entire duration of each session. Common site of stimulation across all patients and montages was represented by the right temporal lobe targeted via T8 (10/20 EEG system). Given the long stimulation sessions and the specific patient population, during tACS participants were instructed to watch a series of pre-selected videoclips from a list of selected documentaries freely available on YouTube, with the aim of maintaining a constant brain state while reducing distraction and avoiding constant interaction with operators in the room. The research team selected videos based on their length (i.e., to be approximately 1 h long), thematic subject (i.e., excluding documentaries related to war or other conflictual subjects that could cause excessive arousal and activation in the participants), and language (i.e., excluding those with extremely technical/specific terms). The themes were counterbalanced across genres to provide a nice selection of videos that would engage participants and focused their attention (primary goal of the videoclips) and be palatable for patients with diverse preferences (e.g., documentaries on animals, nature, history, technology, as well as on filmmaking and music). Each day patients were offered to choose from the list, or resume the video presented during the previous tACS session.

Biophysical modeling

Given the expected variability in cortical atrophy among participants, we did not use a fixed montage (electrode positions and currents) across participants of Group 1, but instead, we defined a cortical target designed to keep normal electrical field (En-field) amplitude fixed on the highest Aβ deposition areas, seeking to ensure that participants received a similar electric field dose. Some variability was still observed due to the constraints on the currents that the stimulator can output and the different sizes of the targets. The resulting montage included 8 stimulation electrodes, delivering tACS at 40Hz with a maximum intensity of 2mA on each electrode according to current tACS safety guidelines [36], and with a resulting higher induced field on the temporal lobe due to Aβ distribution. In Group 1, tACS was not in-phase for all electrodes since obtaining in-phase only stimulation would not be possible (current conservation). The target phase for each area was optimized so that the induced field would be maximal on the PET-defined targets, leading to an inter-region 180 phase choice. The approach for identifying optimal stimulation targets by fusing PET and MRI data for each patient was developed by the PI of the studies (ES); personalized montages were computed by the PI in collaboration with the Neuroelectrics team using the Stimviewer algorithm and the methods described in [37, 38], adapted to the case of tACS.

The same safety guidelines were followed for the stimulation templates used for Group 2 and Group 3, but stimulation was targeted over the bilateral temporal lobes via 4 fixed stimulating electrodes (P8, T8, P7, and T7, right electrodes delivering current with a 180° phase degree respect to the left ones), given the usual pattern of deposition of Aβ and tau protein commonly involving the bilateral temporal lobes when patients are symptomatic [34] (Fig. 1B). Electrode locations and stimulation intensity for bitemporal tACS used in Groups 2 and 3 were defined by the PI of the studies (ES). Stimulation intensity was titrated for each patient, given the typical discomfort reported for transcranial electrical stimulation (tES) delivered over the temporal regions. Whole-scalp 64-channel resting-state EEG was collected in the week before and during the week after the tACS treatment course, along with a comprehensive neurocognitive assessment.

MRI scan

Neuroimaging acquisition was performed on a GE 3 Tesla MR750 scanner using a 32-channel head array coil from Nova Medical. Participants underwent high resolution T1-weighted structural scan (3D T1-w BRAVO), two runs of resting state functional connectivity, resting perfusion MRI with ASL, diffusion tensor imaging, T2* GRE, and FLAIR sequences (total scan timing 60 min). ASL studies were performed with 3D pseudo-continuous labeling (1.45s labeling, 2.025s post-labeling delay), background suppression, and 32 centric ordered 4mm thick slices.

ASL preprocessing and analysis

ASL data preprocessing was performed via an ad-hoc pipeline implemented in MATLAB (MATLAB 2016b, MathWorks) and SPM12 (https://www.fil.ion.ucl.ac.uk/spm/) developed by the laboratory of one of the co-authors at BIDMC (DCA, inventor of the pseudo-continuous ASL technique). Segmentation into three classes (grey matter, white matter, and CSF) and normalization of 3D T1w BRAVO images were obtained via DARTEL [39]. ASL subtraction images were co-registered to the grey matter map and normalized to MNI152 space. Normalized CBF maps were masked with Intracranial Volume (ICV) mask from SPM12 and globally normalized for the CBF values. Manual masking of every CBF map with the

corresponding normalized grey matter mask obtained during the segmentation process was performed for each patient for the two timepoints (pre-stimulation and post-stimulation). Grey matter CBF maps were smoothed with a full width at half maximum (FWHM) of 6 mm.

In order to check for perfusion MRI data quality and check that that participants aligned the expected values of CBF in the temporal lobes (usually around 30 mL/min/100g for grey matter in AD patients [3, 5]), individual CBF values from the temporal lobes were extracted using the REX toolbox (https://www.nitrc.org/projects/rex/) embedded in SPM12. Longitudinal statistical analyses assessing the impact of tACS were conducted in SPM12 on normalized grey matter CBF maps via a paired *t* test on all participants ($n = 15$; single voxel level $p<0.001$, cluster-level $p<0.05$, FDR corrected) as well as on each group of participants separately. Analyses were carried out at whole-brain level, to ensure observed changes in CBF were not amplified by the selection of a specific region-of-interest. In the case a significant change in perfusion was identified, CBF changes within significant clusters were correlated with changes (Δ = post minus pre) at episodic memory and language tests, as well as changes in gamma spectral power after tACS measured via EEG. Finally, the SPM Anatomy toolbox was used to label significant clusters extracted via SPM via a probabilistic atlas (see Table 1).

EEG recording and analysis

Whole-scalp 64-channel resting-state EEG was collected in the week before and in the week after the tACS treatment course via an actiCHamp EEG amplifier system (Brain Products GmbH). EEG recording was obtained while subjects sat in a semi-reclined armchair. During recordings, participants were instructed to remain quiet with their face muscles relaxed. Given the specific study population, particular care was put into ensuring participants understood the importance of staying still and quiet during recording. Both participant and EEG were monitored for signs of drowsiness, at which point the participant was asked to blink their eyes a few times and reminded to stay awake. Recording was done at a sampling rate of 1Khz and impedances were maintained below 5 kΩ during recording.

Data were preprocessed using EEGLAB 2020 [40], Fieldtrip toolbox for EEG/MEG-analysis (Donders Institute for Brain, Cognition and Behaviour, Radboud University, the Netherlands, see http://fieldtriptoolbox.org),

Table 1 Significant clusters of changes in perfusion across all subjects. Probability anatomical mapping and cluster coordinates for significant CBF changes detected when analyzing whole-brain cortical CBF changes (post>pre) across all subjects (upper panel), and for Group 3 participants who received the 4 weeks tACS intervention

All subjects			**Cluster #1**		**Anatomical probability mapping**
Local Maxima Subcluster	***t* value**		**MNI**		
		x	***y***	***z***	
#1	t = 4.64	32	18	-40	R Medial Temporal Pole
#2	t = 3.87	28	2	-44	R Fusiform Gyrus
#3	t = 3.18	26	0	-40	R Entorhinal Cortex
#4	t = 2.96	26	8	-36	R Entorhinal Cortex
#5	t = 2.82	26	16	-40	R Medial Temporal Pole
#6	t = 2.69	32	12	-42	R Medial Temporal Pole
#7	t = 2.66	28	10	-42	R Medial Temporal Pole
Group 3			Cluster #1		
#1	t = 14.66	24	6	-40	R Entorhinal Cortex
#2	t = 12.84	28	8	-42	R Medial Temporal Pole
#3	t = 9.36	26	12	-44	R Fusiform Gyrus
#4	t = 8.54	30	10	-46	R Fusiform Gyrus
#5	t = 8.38	20	8	-40	R Medial Temporal Pole
#6	t = 7.67	28	18	-40	R Medial Temporal Pole
Group 3			Cluster #2		
#1	t = 4.83	-30	-2	-34	L Fusiform Gyrus
#2	t = 4.18	-26	-6	-30	L ParaHippocampal Gyrus
#3	t = 3.77	-28	-14	-26	L Subiculum
#4	t = 3.66	-32	-14	-26	L CA1 (Hippocampus)
#5	t = 3.39	-30	-10	-26	L CA1 (Hippocampus)

the Brainstorm suite [41], and in-house scripts in Matlab R2017b (MathWorks Inc.). Data were initially reduced into 60 dimensions by using principal component analyses (PCA) to minimize overfitting and noise components. Band pass filter was performed using a forward-backward 4th order Butterwoth filter from 1 to 100Hz, a notch filter between 58 and 62Hz was applied, and the data were subsequently referenced to a global average. Subsequently, independent component analysis (ICA) was run to manually remove all remaining artifact components including eye movement/blink, muscle noise (EMG), single electrode noise, cardiac beats (EKG), as well as auditory evoked potentials. Finally, the data were interpolated for missing/removed channels using a 32 spherical interpolation.

Given the longitudinal CBF changes involving primarily the bilateral temporal lobes and the right anterior temporal lobe in particular, changes in gamma spectral power were focused on an array of tACS electrodes indexing the bilateral temporal lobes (i.e., T8, P8, P7, T7), as well as on electrode T8 as a proxy to the right anterior temporal lobe and the common stimulation electrode across tACS montages. Moreover, considering the documented slowing of EEG activity in AD patients [16, 42], with increasing spectral power for activity in the theta and delta band associated with a decrease of fast oscillations such as beta and gamma, statistical analysis was centered on detecting potential changes in gamma spectral power as well as signs of a change in spectral frequency distribution (e.g., restoration of gamma and/or decrease of slower oscillatory activity). Also, considering the limited sample size and exploratory nature of the study, we opted for a simpler statistical framework rather than a full-blown repeated measured ANOVA. Specifically, longitudinal changes in each frequency band were quantified by subtracting baseline absolute spectral power values from post-tACS ones (e.g., baseline theta minus post-tACS theta). A one-way ANOVA with a single factor "Frequency" was computed comparing the pre-posts differences in each frequency band (alpha level = 0.05). Once a main effect was found, post hoc comparisons between pairs of frequency bands were computed as well. EEG bands were defined as follows: delta (1–4 Hz), theta (4–8 Hz), alpha (9–13 Hz), beta (14–30 Hz), low gamma (35–45 Hz), narrow gamma (38–42Hz; centered around the stimulation frequency of 40Hz), mid gamma (45–60 Hz), and high gamma (60–90 Hz). One participant of Group 2 did not complete the post-tACS EEG assessment; analyses were conducted on 14 participants.

Cognitive assessment

Participants underwent specific tests evaluating global cognition (Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog) [43]; MMSE [44], Montreal Cognitive Assessment (MoCA) [45], activities of daily living (ADL) [46]) to assess any potential change in overall cognitive functioning after tACS. Additionally, tasks assessing cognitive functions relevant for the brain regions stimulated by tACS were also used, using the National Alzheimer's Coordinating Center Uniform Data Set (NACC UDS) Neuropsychological Battery: the Craft Story 21 Recall Immediate and Delayed addressing episodic memory [47], and the Category Fluency task (animals), a widely used measure of verbal fluency and language [48].

Results

All 15 participants completed the study and tolerated the intervention with only minor side effects commonly reported in the tACS literature: tingling (10/15) rated as mild; scalp irritation (7/15) rated as mild-moderate; visual changes (8/15) rated as mild-moderate, and headache (5/15, rated as mild-moderate) induced by mechanical pressure from the stimulation cap. Participants attended 95% of the study visits (190/200 daily tACS visits, 10 sessions in total missed distributed across 7 patients), showing excellent treatment compliance. No epileptiform alterations were detected at the electrophysiological as well as clinical assessments.

Perfusion changes

Mean CBF values in the right temporal lobe calculated at baseline (pre tACS intervention) across all the participants were 32.3 mL/min/100g (SD = 6.8), consistent with literature on hypoperfusion in AD patients [3, 5] and validating image acquisition and CBF extraction procedures. Post intervention CBF values in the right temporal lobe increased significantly from 32.3 to 34 mL/min/100g (SD = 8.5) ($t = 2.01$, $p < 0.05$; Cohen's $d = 0.22$). CBF values of the left temporal lobe were extracted for patients of Group 2 and 3 (n=10) who received bitemporal tACS, showing a mean value of 33 mL/min/100g (SD = 6.5) at baseline and 34 mL/min/100g (SD = 6.4) after the intervention ($t = 1.24$, $p = 0.11$). CBF values of the right frontal lobe were extracted for Group 1 that received personalized right temporo-frontal stimulation, revealing a baseline CBF of 35.5 mL/min/100g (SD = 9.7) and 39 mL/min/100g (SD = 14) after the intervention (t =2.36, p = 0.35; Cohen's $d = 0.29$).

Apart from standard regional CBF assessment, voxel-wise whole brain analyses with no prespecified masks were performed to guarantee a more unbiased result. When comparing post-tACS CBF maps with pre-tACS CBF maps across the entire brain in all subjects, a significant CBF increase was detected in multiple anatomical clusters primarily located in the right temporal

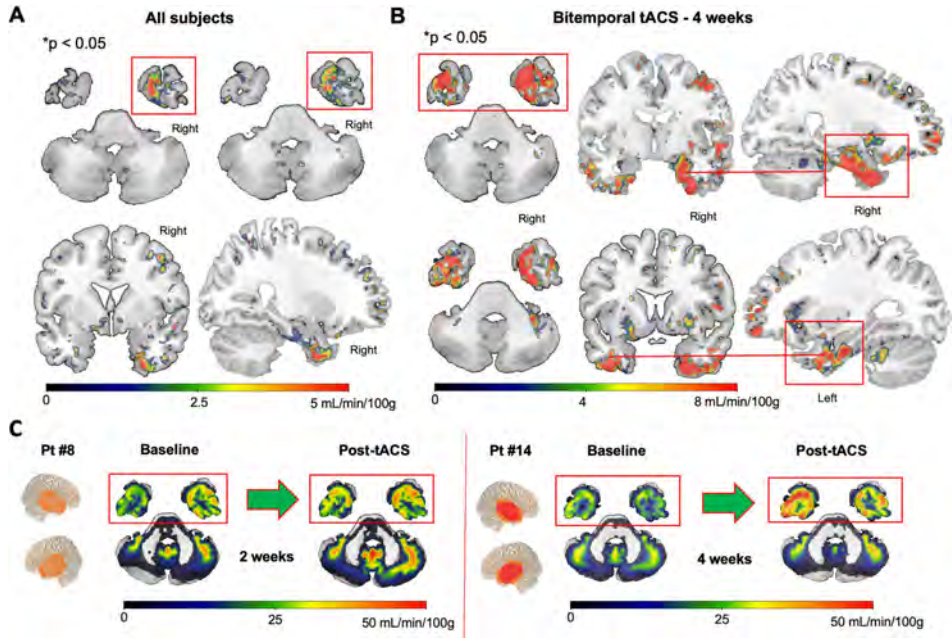

Fig. 2 Perfusion results. **A** CBF increase after tACS. Paired t test (post>pre, $p<0.05$, FDR-corrected) revealed an increase of CBF selectively involving the right temporal lobe, representing the common site of stimulation across participants ($n = 15$). **B** Whole cortical brain CBF analyses of participants who received the highest dose of tACS (20h of bilateral temporal lobe stimulation over 4 weeks, $n = 5$, Group 3) revealed a selective increase in CBF in the bilateral temporal lobes, accordingly to the stimulation template ($p<0.05$, FDR-corrected). **C** Examples of CBF variations in two representative participants belonging to Group 2 (pt #8) and 3 (pt #14)

lobe (n=15, Fig. 2A) ($p<0.05$, FDR-corrected), specifically involving the right medial temporal pole, fusiform gyrus, and entorhinal cortex (see Table 1 for probability anatomical mapping and clusters' coordinate). Of note, results are consistent with the right temporal lobe being the only region consistently stimulated across all 15 participants.

Dividing participants on the basis of their respective tACS montage and looking at pre-post tACS CBF changes, both temporo-frontal (Group 1) and bitemporal (Groups 2 and 3) tACS montages resulted in significant CBF changes, however displaying different topographies of local CBF increase reflecting the two montages: Group 1 (right temporo-frontal tACS; Fig. 3A); Group 2 − 3 (bitemporal tACS; Fig. 3B).

Additionally, when looking specifically at Group 3 who received the longest intervention targeting the bilateral temporal lobes (i.e., 20 h of tACS over 4 weeks compared to 10h in Group 1 and 2), a significant pattern of CBF increase mainly involving the bilateral temporal lobes was found, including medial temporal poles, fusiform gyri, bilateral entorhinal cortices and hippocampi (see Table 1 for probability anatomical mapping and clusters' coordinate), matching the bitemporal tACS montage ($t = 2.13$, $p<0.05$, FDR-corrected; Fig. 2B). For more detailed results on CBF changes in the left and right temporal lobe please see Supplementary Materials.

Post-tACS EEG changes

The One-way ANOVA on T7/8-P7/8 cluster, corresponding to the bitemporal stimulation, revealed an effect of tACS on spectral power around the stimulation frequency (i.e., narrow gamma, 38–42Hz), compared to the rest of the spectrum ($F_{(7,78)} = 4.12$, $p < 0.05$; $\eta 2 = 0.03$) (Fig. 4). Post hoc analysis showed that narrow gamma spectral power displays a post tACS increase higher than activity in the theta band ($t = 3.43$, $p < 0.01$ Cohen's $d = 0.031$), beta band ($t = 2.37$, $p < 0.05$; Cohen's $d = 0.026$) and high gamma band ($t = 1.84$, $p < 0.05$ Cohen's $d = 0.024$) (Fig. 4A). The same analysis performed on

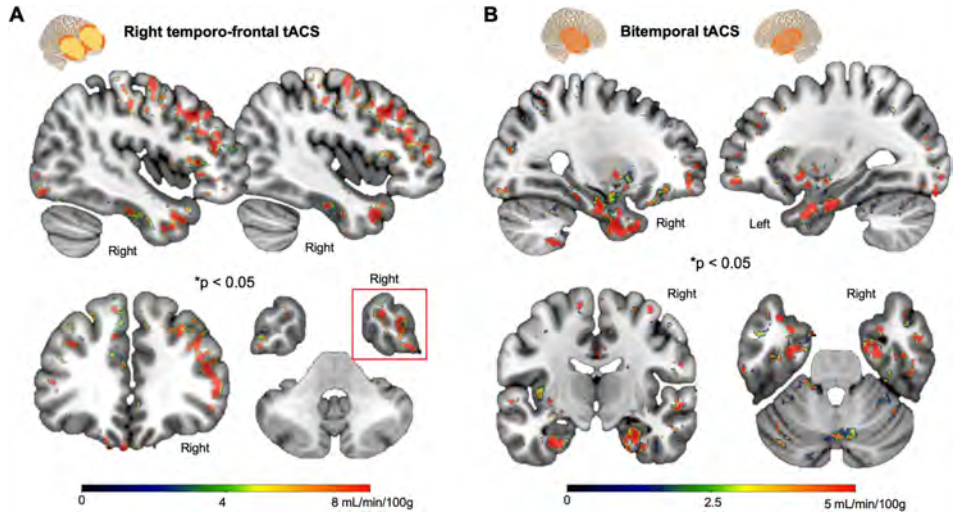

Fig. 3 Comparison of CBF increases between tACS montages. **A** When looking at significant CBF increase after tACS in Group #1 of participants who received right temporo-frontal stimulation, a pattern of predominant right temporo-frontal CBF increases was found, matching with tACS targeting. **B** CBF increase in participants from Group #2 + #3 ($n = 10$) who received bilateral temporal lobe stimulation showed a significant increase of CBF predominantly localized in bilateral temporal regions

electrode T8, the common site of stimulation across groups, produced a similar distribution of pre-post tACS changes across frequencies, with a significant difference between post tACS changes in the narrow gamma and theta bands ($t = 2.06$, $p < 0.05$; Cohen's d 0.025). Even though other gamma frequency sub-bands displayed a similar trend of narrow gamma, no other comparisons reached significance.

Narrow gamma spectral power changes observed on T8 were found to significantly correlate with increase in CBF in the right anterior temporal lobe. In details, narrow gamma spectral power changes on T8 significantly correlated with the cluster of significant CBF increase extracted among all available participants (total = 12; one participant did not complete the post tACS EEG assessment, 2 outliers were removed) ($r = 0.57$; $p = 0.05$; R^2= 33% Fig. 4B).

Cognition and perfusion longitudinal correlation

No significant changes ($p > 0.05$) in overall cognition were found after tACS (ADAS-Cog baseline mean = 18.27, SD = 7.68, post = 18.11, SD = 7.69, Cohen's d = 0.02; ADL baseline mean = 68.5, SD = 4.68, post = 68.3, SD = 6.23, Cohen's d = 0.03; MMSE baseline mean = 23.53, SD = 3.35, post = 22.77, SD: 3.68, Cohen's $d = 0.21$; MoCA: baseline mean = 15.73, SD = 4.23, post = 17.53, SD: 4.5, Cohen's $d = 0.41$). Changes at memory and language tests did not reach significance ($p > 0.05$): Craft Story Recall - Immediate (mean pre-tACS *Verbatim* = 8.13, SD = 3.71; mean post-tACS = 7.93, SD = 5.83, Cohen's $d = 0.04$), and *Paraphrase* (mean pre-tACS Paraphrase = 6.93, SD = 3.77; mean post-tACS = 6.80, SD = 4.1, Cohen's $d = 0.03$); Craft Story Recall - Delayed (mean pre-tACS *Verbatim* = 3.87, SD = 3.64, mean post-tACS = 5.20, SD = 5.43, Cohen's $d = 0.28$; mean pre-tACS *Paraphrase* = 3.87, SD = 3.35, mean post-tACS = 4.93, SD = 4.3, Cohen's d = 0.27); Category Fluency (animals, total correct, mean pre-tACS = 11.47, SD = 5.43; mean post-tACS = 11.33, SD = 4.6, Cohen's $d = 0.02$).

Significant CBF changes positively correlated with changes at Craft Story Recall - Delayed (Fig. 4C). In detail, ΔCBF value (post tACS CBF minus pre tACS CBF) extracted from the regions displaying a significant longitudinal CBF change after tACS showed a positive correlation with changes (post minus pre) at Craft Story Recall - Delayed Verbatim ($r = 0.53$, $p = 0.04$, R^2 = 0.29) and Paraphrase ($r = 0.60$, $p = 0.01$, $R^2 = 0.36$) (Fig. 4C). Additional correlations with memory/fluency scores using CBF values extracted separately from the

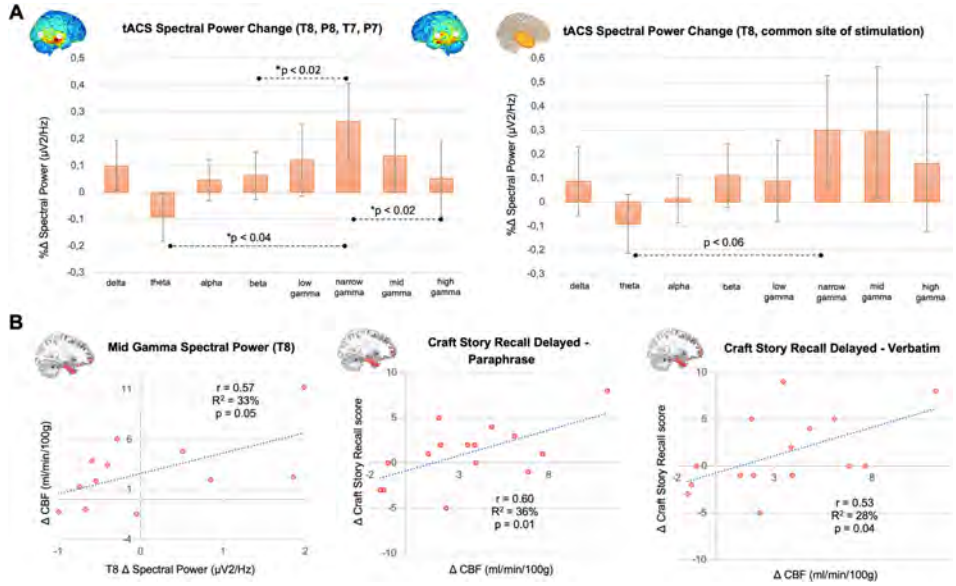

Fig. 4 CBF changes, EEG results and correlations with cognition. **A** Changes in spectral power in the gamma band for a cluster of EEG electrodes indexing regions displaying post-tACS increase in perfusion (T8, P8, P7, T7; left panel) are reported, as well as for the electrode T8 (right panel) representing the common tACS electrode across all participants and the scalp electrode more proximal to the right anterior temporal lobe displaying the highest change in CBF post-tACS. **B** Spectral power changes in the narrow gamma band (38-42 Hz) detected on T8 significantly correlate with CBF changes in the right temporal lobe (left panel). Significant CBF variations also showed a significant correlation with variations in memory performance scores pre-post tACS. Specifically, CBF variation in the right temporal regions across all participants (n=15) positively correlated with performance changes at both paraphrase (mid panel) and verbatim (right panel) recollection components of an episodic memory task

left and right temporal lobes are reported in the Supplementary Materials.

Discussion

Our data suggest that repetitive sessions of gamma tACS lead to a significant increase in CBF in the temporal lobes, without adverse effects. Specifically, when analyzing whole-brain cortical CBF across all participants, a significant increase was revealed in the right temporal lobe, the region consistently stimulated across all participants, also including the entorhinal cortex (Fig. 2). Moreover, when restricting the analysis to participants receiving bilateral temporal lobe stimulation for 4 weeks (the highest tACS dose), a significant increase in CBF was observed in both left and right temporal lobes, including their mesial parts as well as the hippocampi (Fig. 2). Preliminary evidence of tACS target engagement specificity was observed when comparing perfusion changes obtained with two different stimulation templates (Fig. 3). Finally, gamma spectral power changes were found to be correlated to CBF increase (Fig. 4), as well as moderately correlated with changes in cognitive performance related to episodic memory and fluency (Fig. 4 and Figure S1), two domains loading on the temporal lobe and commonly impaired in AD patients. Findings open up potentially interesting avenues for AD patients and other conditions characterized by hypoperfusion—even though the causal role of hypometabolisms/perfusion in the AD pathophysiological cascade is still unclear—as well as for potential effects related to recent preclinical evidence of gamma-mediated amyloid and tau clearance.

To the best of our knowledge, only two studies have reported an increase in regional CBF in AD patients receiving a drug treatment (e.g., donepezil) [49, 50]. Present preliminary results support the recent growing relevance of gamma activity in AD pathophysiology, while at the same time offering a potential evidence of the recently documented causal role of gamma band activity on vessel diameter variation in the human brain [11]. While gamma activity has been discovered to be a driver

of arteriolar vasomotion in animals, the molecular/biological mechanisms translating neuronal spiking into arteriolar diameter variations have not been univocally clarified yet [11]. The most recent observations coming from studies investigating tES in preclinical models suggest the possibility to induce (i) an immediate, primary vasodilatory response via perivascular neurons-mediated and endothelial-mediated pathways (e.g., acting on dural/pial arteries and penetrating arterioles targeted by current flowing in perivascular parasympathetic nerves that mainly cause vasodilatation), and (ii) an indirect, secondary vascular effect via neurovascular coupling, with the involvement of astrocytes and neurons [51–53]. Of note, tES could cause both primary and secondary response by acting on the same target. For instance, pericytes—the cells wrapped around the endothelial wall of capillaries contributing to form the neurovascular unit—regulate the arteriolar and capillary diameter vessels in response to regional neuronal activity [54]. It could be possible that tACS also leads to an indirect modulation of pericytes, as well as astrocytes, as a consequence of direct neuronal modulation, apart from a modulation of peptides released by the stimulated cells themselves [51, 55].

At the pathophysiological level, CBF variations are a consequence of changes in brain glucose metabolism, and a decrease in CBF is thought to reflect synaptic failure [56–58]. Indeed, loss of synapses is considered the most important and direct phenomenon underpinning cognitive decline, ultimately responsible for network disruption [16, 59]. Within this framework, 40Hz tACS could be tackling the AD pathophysiological cascade by modulating interneuron activity contributing to global network dysfunction and by activating microglia waste removal [16], and/or by restoring perfusion in impaired cortical areas to guarantee an adequate amount of nutrients and clearance of toxic products, also given the arteriolar contribution to the glymphatic system pathway [14]. Interestingly, tau pathology has been recently associated with hypoperfusion in the entorhinal cortex, even if the exact underlying pathophysiological mechanism remains to be clarified [60]. Finally, other neuropsychiatric diseases share the cellular substrates of impaired metabolism and reduced interneuron activity observed in AD, in particular frontotemporal dementia (FTD) [16], schizophrenia [61], and autism spectrum disorder [62], suggesting tACS could also benefit these patient populations (e.g., see NCT04425148, 40Hz tACS in FTD).

As for the location of CBF changes and dose-response effects of tACS, good spatial specificity was observed in our data with a primary involvement of the temporal lobes. Previous studies have revealed that alterations in gamma activity over the entorhinal-hippocampal circuit in AD mouse models cause memory impairments [63], and there is ample evidence that gamma and theta oscillations, as well as their phase reciprocal relationship, are crucial for memory processes in general [64]. In particular, gamma oscillations are prominently and physiologically expressed by the entorhinal-hippocampal circuit, potentially making the probability of inducing gamma entrainment via tACS more plausible in these regions, even in the presence of an underlying pathologically desynchronized gamma activity [63]. Further studies with larger samples of participants are needed to identify the most optimal treatment protocols in terms of dose-response effects (i.e., 1 week of daily stimulation followed by rest, 4 weeks of continuative treatment). It must be noticed that high resolution 64-channels EEG recording was performed as part of the baseline and follow-up assessments taking place before and after the entire treatment course. Specifically, the two study visits were prioritized so that they would happen right before (i.e., on a Friday before starting the tACS treatment on the subsequent Monday) and right after the treatment (i.e., on a Monday after the last week of treatment). However, logistical issues related to scheduling of the remaining study visits (e.g., MRI, PET), as well as patients' availability, sometimes interfered with the originally planned schedule. Therefore, a delay assessment of gamma oscillatory activity post-treatment was present, making the observed changes in gamma spectral power more likely to represent a hint to long-lasting tACS after effects rather than acute changes in brain oscillatory activity. Future studies should include a longitudinal EEG assessment covering multiple time points starting from the end of the last tACS sessions in order to properly characterize individual trajectory of tACS effects.

Even though short as compared to drug trials, the tACS treatment was longer than any publicly available protocol in AD patients or healthy controls—with a maximum of 20h of stimulation over 4 weeks, thus corroborating the safety profile of tACS as well as its feasibility in patients with AD, with no adverse events and strong adherence to the treatment regimen. At the same time, the relatively short duration of the intervention with respect to pharmacological trials (e.g., 6–12 months [65]) could be responsible for the lack of significant changes on global scales of cognition after the intervention, along with the limited sample size. Indeed, a trend for improvement at the MoCA test, known to be able to detect subtle cognitive changes especially in the mild dementia phase, was found (baseline mean = 15.73, SD = 4.23, post = 17.53, SD: 4.5), with a moderate effect size (Cohen's d = 0.41). This result, along with the observed correlations between post-tACS perfusion and episodic memory changes, support the need for longer trials with a bigger sample size to properly evaluate the potential therapeutical effects of

tACS and disentangle the relationship between changes in gamma activity, brain perfusion, and cognitive performance. Home-based tACS delivery should also be considered [66], promoting accessibility to patients and lower burden for caregivers.

Finally, in the present trials, we focused on patients with mild to moderate dementia due to AD given their documented profile of gamma alterations, hypoperfusion, and proteinopathy, allowing to observe potential effects of tACS. However, recent evidence shows how approximately 15–20 years before the onset of cognitive deficits, amyloid starts accumulating, followed by microgliosis and neurofibrillary tangle tau pathology [67], making mild to moderate dementia a relatively advanced stage of AD where significant irreversible neuronal and synaptic loss has occurred and therapeutic countermeasures are likely to be less effective [67]. However, if proven effective, tACS could play a role in earlier stages of the disease, as well as in prodromal AD (e.g., patients with autosomal dominant mutations in the precursor of Aβ or Presenilin), and MCI patients. Given its safety profile and portability, the potential application of tACS as a preclinical preventative intervention aimed at the delay the onset of cognitive manifestations and/or slowing down the course of the disease should be explored.

Limitations

The trials were intended as pilots and, given the limited sample of participants along with the multiple targeting approaches, did not aim to offer definitive answers on any aspect of the study. A larger sample is needed to confirm the observed increase in CBF as well as changes in gamma spectral power, possibly by reducing the number of daily visits performed at the hospital (e.g., using home-based tACS; present pilots consisted on a total of 200 daily tACS sessions and approximately 240 baseline/follow-ups study visits) and by simplifying the study design in general. A control tACS condition, including sham (placebo) stimulation and potentially a control stimulation frequency, should be included as well (NCT03880240) to ensure that the observed CBF increase is related to the tACS intervention rather than to an unspecific effect of exposure to the study (e.g., daily interaction with health care providers), even if unlikely in the light of the spatial specificity of the effect and the unlikeliness of a spontaneous focal perfusion increase in mild to moderate AD patients.

Conclusions

Present findings promote a framework for the investigation of tACS-based interventions to increase brain perfusion in AD patients, showing preliminary evidence of the impact of 40Hz tACS on local CBF in the temporal lobe, entorhinal cortex, and hippocampi.

Abbreviations

AD: Alzheimer's disease; ADAS-cog: Alzheimer's Disease Assessment Scale-Cognitive Subscale; ADL: Activities of daily living; ASL: Arterial spin labeling; CBF: Cerebral blood flow; EEG: Electroencephalography; FTD: Frontotemporal dementia; MCI: Mild cognitive impairment; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; MRI: Magnetic resonance imaging; NACC UDS: National Alzheimer's Coordinating Center Uniform Data Set Neuropsychological Battery; PET: Positron emission tomography; PV+ interneurons: Parvalbumin positive interneurons; SPECT: Single-photon emission computed tomography; tACS: ranscranial alternating current stimulation (tACS).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-021-00922-4.

Additional file 1: Supplementary Results. **Figure S1**. Covariation between changes in temporal CBF and memory and language tasks.

Acknowledgments

The authors would like to thank patients and caregivers for their support, and also BIDMC and DARPA (HR001117S0030) for their support to the studies.

Authors' contributions

GS: Formal analysis, Software, Writing - original draft, Writing - review and editing. FM: Formal analysis, Software, Writing - review and editing. DC: Investigation, Formal analysis, Writing - review and editing. RP: Project administration, Investigation, Writing - review and editing. JM: Project administration, Supervision, Writing - review and editing. AC: Project administration, Supervision, Writing - review and editing. GE-F: Investigation, Methodology, Resources, Supervision, Validation, Writing - review and editing. RS: Software, Writing - review and editing. GR: Software, Writing - review and editing. KD: Investigation, Software, Writing - review and editing. MMS: Supervision, Writing - review and editing; Daniel Press: Supervision, Writing - review and editing. DCA: Software, Supervision, Writing - review and editing. APL: Conceptualization, Funding acquisition, Writing - review and editing. ES: Conceptualization, Formal analysis, Software, Funding acquisition, Supervision, Writing - original draft, Writing - review and editing. The authors read and approved the final manuscript.

Funding

This work was supported by the Chief Academic Officer (CAO) from the Beth Israel Deaconess Medical Center (BIDMC) Award 2017 (ES) and by the Defense Advanced Research Projects Agency (DARPA) via HR001117S0030 (ES).

Availability of data and materials

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Declarations

Ethics approval and consent to participate

All participants gave written informed consent prior to participating in the studies, registered separately on ClinicalTrials.gov (NCT03412604, NCT03290326; PI Santarnecchi). The research proposal and associated methodologies were approved by the local ethics committee (Beth Israel Deaconess Medical Center IRB) in accordance with the principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests
DCA is the inventor of the pseudo-continuous ASL technique employed for perfusion MRI in this work. He receives post-market royalties through his institution from licenses to GE Healthcare, Philips Healthcare, Hitachi Medical, Siemens Healthineers, and UIH America. GR is co-founder of Neuroelectrics and RS is an employer. ES and APL are listed co-inventors on an issued patent on the use of tACS in AD. All other authors declare they have no competing interests.

Author details
[1]Berenson-Allen Center for Non-Invasive Brain Stimulation, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA. [2]Department of Radiology, University Hospital of Parma, Parma, Italy. [3]Department of Radiology, Beth Israel Deaconess Medical Center, Boston, MA, USA. [4]Center for Advanced Medical Imaging Sciences, Division of Nuclear Medicine and Molecular Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA. [5]Neuroelectrics, Barcelona, Spain. [6]Hinda and Arthur Marcus Institute for Aging Research and Deanna and Sidney Wolk Center for Memory Health, Hebrew Senior Life, Boston, MA, USA. [7]Department of Neurology, Harvard Medical School, Boston, MA, USA. [8]Guttmann Brain Health Institute, Barcelona, Spain. [9]Precision Neuroscience & Neuromodulation Program, Gordon Center for Medical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

Received: 14 July 2021 Accepted: 20 October 2021
Published online: 20 December 2021

References

1. Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. Neurology. 2013;80:1778–83.
2. Forester BP, Patrick RE, Harper DG. Setbacks and opportunities in disease-modifying therapies in alzheimer disease. JAMA Psychiatry. 2019;77(1):7–8. https://doi.org/10.1001/jamapsychiatry.2019.2332.
3. Dai W, Lopez OL, Carmichael OT, Becker JT, Kuller LH, Gach HM. Mild cognitive impairment and Alzheimer disease: patterns of altered cerebral blood flow at MR imaging. Radiology. 2009;250:856–66.
4. Alsop DC, Detre JA, Golay X, Günther M, Hendrikse J, Hernandez-Garcia L, et al. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: a consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. Magn Reson Med. 2015;73:102–16.
5. Alsop DC, Detre JA, Grossman M. Assessment of cerebral blood flow in Alzheimer's disease by spin-labeled magnetic resonance imaging. Ann Neurol. 2000;47:93–100.
6. Alsop DC, Casement M, de Bazelaire C, Fong T, Press DZ. Hippocampal hyperperfusion in Alzheimer's disease. Neuroimage. 2008;42:1267–74.
7. Chen W, Song X, Beyea S, D'Arcy R, Zhang Y, Rockwood K. Advances in perfusion magnetic resonance imaging in Alzheimer's disease. Alzheimers Dement. 2011;7:185–96.
8. Huang C-W, Hsu S-W, Chang Y-T, Huang S-H, Huang Y-C, Lee C-C, et al. Cerebral perfusion insufficiency and relationships with cognitive deficits in alzheimer's disease: a multiparametric neuroimaging study. Sci Rep. 2018;8:1541.
9. Binnewijzend MAA, Benedictus MR, Kuijer JPA, van der Flier WM, Teunissen CE, Prins ND, et al. Cerebral perfusion in the predementia stages of Alzheimer's disease. Eur Radiol. 2016;26:506–14.
10. Chao LL, Buckley ST, Kornak J, Schuff N, Madison C, Yaffe K, et al. ASL perfusion MRI predicts cognitive decline and conversion from MCI to dementia. Alzheimer Dis Assoc Disord. 2010;24:19–27.
11. Mateo C, Knutsen PM, Tsai PS, Shih AY, Kleinfeld D. Entrainment of arteriole vasomotor fluctuations by neural activity is a basis of blood-oxygenation-level-dependent "resting-state" connectivity. Neuron. 2017;96:936–948.e3.
12. Drew PJ, Mateo C, Turner KL, Yu X, Kleinfeld D. Ultra-slow oscillations in fMRI and resting-state connectivity: neuronal and vascular contributions and technical confounds. Neuron. 2020;107:782–804.
13. van Veluw SJ, Hou SS, Calvo-Rodriguez M, Arbel-Ornath M, Snyder AC, Frosch MP, et al. Vasomotion as a driving force for paravascular clearance in the awake mouse brain. Neuron. 2020;105:549–561.e5.
14. Nedergaard M, Goldman SA. Glymphatic failure as a final common pathway to dementia. Science. 2020;370:50–6.
15. Iaccarino HF, Singer AC, Martorell AJ, Rudenko A, Gao F, Gillingham TZ, et al. Gamma frequency entrainment attenuates amyloid load and modifies microglia. Nature. 2016;540:230–5.
16. Palop JJ, Mucke L. Network abnormalities and interneuron dysfunction in Alzheimer disease. Nat Rev Neurosci. 2016;17:777–92.
17. Adaikkan C, Middleton SJ, Marco A, Pao P-C, Mathys H, Kim DN-W, et al. Gamma entrainment binds higher-order brain regions and offers Neuroprotection. Neuron. 2019;102(5):929–43.e8. https://doi.org/10.1016/j.neuron.2019.04.011. Epub 2019 May 7.
18. Verret L, Mann EO, Hang GB, Barth AMI, Cobos I, Ho K, et al. Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. Cell. 2012;149:708–21.
19. Babiloni C, Lizio R, Marzano N, Capotosto P, Soricelli A, Triggiani AI, et al. Brain neural synchronization and functional coupling in Alzheimer's disease as revealed by resting state EEG rhythms. Int J Psychophysiol. 2016;103:88–102.
20. Thomson H. How flashing lights and pink noise might banish Alzheimer's, improve memory and more. Nature. 2018;555:20–2.
21. Fröhlich F, McCormick DA. Endogenous electric fields may guide neocortical network activity. Neuron. 2010;67:129–43.
22. Ozen S, Sirota A, Belluscio MA, Anastassiou CA, Stark E, Koch C, et al. Transcranial electric stimulation entrains cortical neuronal populations in rats. JNeurosci. 2010;30:11476–85.
23. Johnson L, Alekseichuk I, Krieg J, Doyle A, Yu Y, Vitek J, et al. Dose-dependent effects of transcranial alternating current stimulation on spike timing in awake nonhuman primates. Sci Adv. 2020;6(36):eaaz2747. https://doi.org/10.1126/sciadv.aaz2747. Print 2020 Sep.
24. Krause MR, Vieira PG, Csorba BA, Pilly PK, Pack CC. Transcranial alternating current stimulation entrains single-neuron activity in the primate brain. Proc Natl Acad Sci U S A. 2019;116:5747–55.
25. Schmidt SL, Iyengar AK, Foulser AA, Boyle MR, Fröhlich F. Endogenous cortical oscillations constrain neuromodulation by weak electric fields. Brain Stimul. 2014;7:878–89.
26. Ahn S, Mellin JM, Alagapan S, Alexander ML, Gilmore JH, Jarskog LF, et al. Targeting reduced neural oscillations in patients with schizophrenia by transcranial alternating current stimulation. Neuroimage. 2019;186:126–36. https://doi.org/10.1016/j.neuroimage.2018.10.056. Epub 2018 Oct 24.
27. Ahn S, Prim JH, Alexander ML, McCulloch KL, Fröhlich F. Identifying and engaging neuronal oscillations by Transcranial alternating current stimulation in patients with chronic low Back pain: a randomized, crossover, double-blind, sham-controlled pilot study. J Pain. 2019;20(3):277.e1–277.e11. https://doi.org/10.1016/j.jpain.2018.09.004. Epub 2018 Sep 27.
28. Antonenko D, Faxel M, Grittner U, Lavidor M, Flöel A. Effects of Transcranial alternating current stimulation on cognitive functions in healthy young and older adults. Neural Plast. 2016;2016:4274127.
29. Santarnecchi E, Biasella A, Tatti E, Rossi A, Prattichizzo D, Rossi S. High-gamma oscillations in the motor cortex during visuo-motor coordination: a tACS interferential study. Brain Res Bull. 2017;131:47–54.
30. Santarnecchi MT, Rossi S, Sarkar A, Polizzotto NR, Rossi A, et al. Individual differences and specificity of prefrontal gamma frequency-tACS on fluid intelligence capabilities. Cortex. 2016;75:33–43.
31. Santarnecchi E, Sprugnoli G, Bricolo E, Costantini G, Liew S-L, Musaeus CS, et al. Gamma tACS over the temporal lobe increases the occurrence of Eureka! Moments. Sci Rep. 2019;9:5778.
32. Santarnecchi E, Polizzotto NR, Godone M, Giovannelli F, Feurra M, Matzen L, et al. Frequency-dependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. Curr Biol. 2013;23:1449–53.
33. Kasten FH, Dowsett J, Herrmann CS. Sustained aftereffect of α-tACS lasts up to 70 min after stimulation. Front Hum Neurosci. 2016;10:245.
34. Pontecorvo MJ, Devous MD, Navitsky M, Lu M, Salloway S, Schaerf FW, et al. Relationships between flortaucipir PET tau binding and amyloid burden, clinical diagnosis, age and cognition. Brain. 2017;140:748–63.
35. Ruffini G, Wendling F, Sanchez-Todo R, Santarnecchi E. Targeting brain networks with multichannel transcranial current stimulation (tCS). Curr Opin Biomed Eng. 2018;8:70–7.

36. Antal A, Alekseichuk I, Bikson M, Brockmöller J, Brunoni AR, Chen R, et al. Low intensity transcranial electric stimulation: safety, ethical, legal regulatory and application guidelines. Clin Neurophysiol. 2017;128:1774–809.
37. Fischer DB, Fried PJ, Ruffini G, Ripolles O, Salvador R, Banus J, et al. Multifocal tDCS targeting the resting state motor network increases cortical excitability beyond traditional tDCS targeting unilateral motor cortex. Neuroimage. 2017;157:34–44.
38. Ruffini G, Fox MD, Ripolles O, Miranda PC, Pascual-Leone A. Optimization of multifocal transcranial current stimulation for weighted cortical pattern targeting from realistic modeling of electric fields. Neuroimage. 2014;89:216–25.
39. Ashburner J. A fast diffeomorphic image registration algorithm. Neuroimage. 2007;38:95–113.
40. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods. 2004;134:9–21.
41. Tadel F, Baillet S, Mosher JC, Pantazis D, Leahy RM. Brainstorm: a user-friendly application for MEG/EEG analysis. Comput Intell Neurosci. 2011;2011:879716. https://doi.org/10.1155/2011/879716. Epub 2011 Apr 13.
42. Claus JJ, Kwa VI, Teunisse S, Walstra GJ, van Gool WA, Koelman JH, et al. Slowing on quantitative spectral EEG is a marker for rate of subsequent cognitive and functional decline in early Alzheimer disease. Alzheimer Dis Assoc Disord. 1998;12:167–74.
43. Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. Am J Psychiatry. 1984;141:1356–64.
44. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12:189–98.
45. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53:695–9.
46. Galasko D, Bennett D, Sano M, Ernesto C, Thomas R, Grundman M, et al. An inventory to assess activities of daily living for clinical trials in Alzheimer's disease. The Alzheimer's disease cooperative study. Alzheimer Dis Assoc Disord. 1997;11 Suppl 2:S33–9.
47. Craft S, Newcomer J, Kanne S, Dagogo-Jack S, Cryer P, Sheline Y, et al. Memory improvement following induced hyperinsulinemia in Alzheimer's disease. Neurobiol Aging. 1996;17:123–30.
48. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The consortium to establish a registry for Alzheimer's disease (CERAD). Part I. clinical and neuropsychological assessment of Alzheimer's disease. Neurology. 1989;39:1159–65.
49. Iizuka T, Kameyama M. Cholinergic enhancement increases regional cerebral blood flow to the posterior cingulate cortex in mild Alzheimer's disease. Geriatr Gerontol Int. 2017;17:951–8.
50. Li W, Antuono PG, Xie C, Chen G, Jones JL, Ward BD, et al. Changes in regional cerebral blood flow and functional connectivity in the cholinergic pathway associated with cognitive performance in subjects with mild Alzheimer's disease after 12-week donepezil treatment. Neuroimage. 2012;60:1083–91.
51. Bahr-Hosseini M, Bikson M. Neurovascular-modulation: a review of primary vascular responses to transcranial electrical stimulation as a mechanism of action. Brain Stimul. 2021;14:837–47.
52. Turner DA, Degan S, Galeffi F, Schmidt S, Peterchev AV. Rapid, dose-dependent enhancement of cerebral blood flow by transcranial AC stimulation in mouse. Brain Stimul. 2020;14:80–7.
53. Zheng X, Alsop DC, Schlaug G. Effects of transcranial direct current stimulation (tDCS) on human regional cerebral blood flow. Neuroimage. 2011;58:26–33.
54. Peppiatt CM, Howarth C, Mobbs P, Attwell D. Bidirectional control of CNS capillary diameter by pericytes. Nature. 2006;443:700–4.
55. Masamoto K, Unekawa M, Watanabe T, Toriumi H, Takuwa H, Kawaguchi H, et al. Unveiling astrocytic control of cerebral blood flow with optogenetics. Sci Rep. 2015;5:11455.
56. Chen Y, Wolk DA, Reddin JS, Korczykowski M, Martinez PM, Musiek ES, et al. Voxel-level comparison of arterial spin-labeled perfusion MRI and FDG-PET in Alzheimer disease. Neurology. 2011;77:1977–85.
57. Jueptner M, Weiller C. Review: does measurement of regional cerebral blood flow reflect synaptic activity? Implications for PET and fMRI. Neuroimage. 1995;2:148–56.
58. Musiek ES, Chen Y, Korczykowski M, Saboury B, Martinez PM, Reddin JS, et al. Direct comparison of fluorodeoxyglucose positron emission tomography and arterial spin labeling magnetic resonance imaging in Alzheimer's disease. Alzheimers Dement. 2012;8:51–9.
59. Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci. 2010;13:812–8.
60. Rubinski A, Tosun D, Franzmeier N, Neitzel J, Frontzkowski L, Weiner M, et al. Lower cerebral perfusion is associated with tau-PET in the entorhinal cortex across the Alzheimer's continuum. Neurobiol Aging. 2021;102:111–8.
61. Seethalakshmi R, Parkar SR, Nair N, Adarkar SA, Pandit AG, Batra SA, et al. Regional brain metabolism in schizophrenia: an FDG-PET study. Indian J Psychiatry. 2006;48:149–53.
62. Kayarian FB, Jannati A, Rotenberg A, Santarnecchi E. Targeting gamma-related pathophysiology in autism Spectrum disorder using Transcranial electrical stimulation: opportunities and challenges. Autism Res. 2020;13(7):1051–71. https://doi.org/10.1002/aur.2312. Epub 2020 May 28.
63. Nakazono T, Jun H, Blurton-Jones M, Green KN, Igarashi KM. Gamma oscillations in the entorhinal-hippocampal circuit underlying memory and dementia. Neurosci Res. 2018;129:40–6.
64. Düzel E, Penny WD, Burgess N. Brain oscillations and memory. Curr Opin Neurobiol. 2010;20:143–9.
65. Takeshima N, Ishiwata K, Sozu T, Furukawa TA. Primary endpoints in current phase II/III trials for Alzheimer disease: a systematic survey of trials registered at ClinicalTrials.Gov. Alzheimer Dis Assoc Disord. 2020;34:97–100.
66. Sandran N, Hillier S, Hordacre B. Strategies to implement and monitor in-home transcranial electrical stimulation in neurological and psychiatric patient populations: a systematic review. J Neuroeng Rehabil. 2019;16:58.
67. Long JM, Holtzman DM. Alzheimer disease: an update on pathobiology and treatment strategies. Cell. 2019;179:312–39.

Publisher's Note

frontiers in Neurology

ORIGINAL RESEARCH
published: 20 May 2021
doi: 10.3389/fneur.2021.598135

Patient-Tailored, Home-Based Non-invasive Brain Stimulation for Memory Deficits in Dementia Due to Alzheimer's Disease

Lucie Bréchet[1,2], *Wanting Yu*[1], *Maria Chiara Biagi*[3], *Giulio Ruffini*[3,4], *Margaret Gagnon*[1], *Brad Manor*[1,5] *and Alvaro Pascual-Leone*[1,2,6*]

[1] Hinda and Arthur Marcus Institute for Aging Research and Center for Memory Health, Hebrew SeniorLife, Boston, MA, United States, [2] Department of Neurology, Harvard Medical School, Boston, MA, United States, [3] Neuroelectrics Barcelona, Barcelona, Spain, [4] Neuroelectrics Corp., Cambridge, MA, United States, [5] Department of Medicine, Harvard Medical School, Boston, MA, United States, [6] Guttmann Brain Health Institute, Institut Guttman de Neurorehabilitació, Barcelona, Spain

OPEN ACCESS

Edited by:
Francesco Di Lorenzo,
Santa Lucia Foundation (IRCCS), Italy

Reviewed by:
Alberto Benussi,
University of Brescia, Italy
Elissaios Karageorgiou,
Independent Researcher,
Athens, Greece

***Correspondence:**
Alvaro Pascual-Leone
apleone@hsl.harvard.edu

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: 23 August 2020
Accepted: 20 April 2021
Published: 20 May 2021

Citation:
Bréchet L, Yu W, Biagi MC, Ruffini G, Gagnon M, Manor B and Pascual-Leone A (2021) Patient-Tailored, Home-Based Non-invasive Brain Stimulation for Memory Deficits in Dementia Due to Alzheimer's Disease. Front. Neurol. 12:598135. doi: 10.3389/fneur.2021.598135

Alzheimer's disease (AD) is an irreversible, progressive brain disorder that can cause dementia (Alzheimer's disease-related dementia, ADRD) with growing cognitive disability and vast physical, emotional, and financial pressures not only on the patients but also on caregivers and families. Loss of memory is an early and very debilitating symptom in AD patients and a relevant predictor of disease progression. Data from rodents, as well as human studies, suggest that dysregulation of specific brain oscillations, particularly in the hippocampus, is linked to memory deficits. Animal and human studies demonstrate that non-invasive brain stimulation (NIBS) in the form of transcranial alternating current stimulation (tACS) allows to reliably and safely interact with ongoing oscillatory patterns in the brain in specific frequencies. We developed a protocol for patient-tailored home-based tACS with an instruction program to train a caregiver to deliver daily sessions of tACS that can be remotely monitored by the study team. We provide a discussion of the neurobiological rationale to modulate oscillations and a description of the study protocol. Data of two patients with ADRD who have completed this protocol illustrate the feasibility of the approach and provide pilot evidence on the safety of the remotely-monitored, caregiver-administered, home-based tACS intervention. These findings encourage the pursuit of a large, adequately powered, randomized controlled trial of home-based tACS for memory dysfunction in ADRD.

Keywords: transcranial alternating current stimulation, Alzheimer's disease, memory improvement, home-based treatment, stimulation modeling, translational science, parietal cortex, angular gyrus

INTRODUCTION

Memories fundamentally shape who we are. The loss of memory has a profound impact on the patient but also on spouses, family, and friends. Currently, there are neither effective interventions nor approved disease-modifying therapies that can effectively address memory deficits in patients with dementia. Cognitive science and neuroimaging studies have investigated memory extensively and identified a core network in the brain that supports memory function and comprises the

hippocampus, medial prefrontal cortex, and angular gyrus (1–3). Activation of these structures seems crucial to retrieve the conscious experience of one-self in the past, and damage to this network can have profound consequences to everyday life (4). Consistently with the discovery of severely amnesic patients following damage to the medial temporal lobe (MTL) (5), many fMRI studies have established the essential role of MTL structures in memory (3, 6). Growing evidence from clinical studies of patients with lesions in the angular gyrus shows that the lateral parietal cortex is also critical for the subjective, conscious experience of retrieving vivid, vibrant, and multisensory (e.g., auditory and visual) episodic memories (7). A deeper understanding of the neurobiological substrate of memory could enable the development of early diagnostic indicators of dementia and novel therapeutic approaches to minimize cognitive disability.

We hypothesize that knowledge about the brain structures and brain network involved in memory encoding and retrieval is necessary but not sufficient to decode the underlying neurobiological mechanism. Specifically, we contend that brain oscillations, which arise from synchronized interactions between neuronal populations, are essential for memory processes. Previous electrophysiological studies have related episodic memory processes to specific oscillatory signatures. Theta oscillations (4–8 Hz) have been identified as the dominant rhythms recorded in the hippocampus, the critical structure for memory formation (8). It has been hypothesized that hippocampal theta not only serves as the binding mechanism for spatial navigation (place cells) but also for forming episodic memory, due to the overlapping nature of brain networks supporting navigation, remembering the past and thinking about the future (9). Apart from theta oscillations, gamma oscillations (25–100 Hz) have also been proposed as a prime candidate for memory-related synaptic changes in the hippocampus (10). A consistent finding in AD patients is a relative attenuation of gamma frequency (11) and dysregulation of gamma activity is also seen in animal models of AD (12). While theta and gamma oscillations in the hippocampus play a central role for episodic memory, the coupling between hippocampal theta activity and neocortical gamma oscillations seems to be the underlying mechanism that supports the interactions within the large-scale memory network (13). These studies provide essential insights into the roles of brain oscillations in memory impairment.

A recent seminal study in a mouse model of AD (5XFAD) found that optogenetic modulation of parvo-albumin positive (PV+) and somatostatin-expressing (SST+) interneurons can restore hippocampal network 40 Hz gamma oscillations and synaptic plasticity (14). Similar results could be induced by multisensory stimulation (15). Remarkably, induction of gamma oscillations via optogenetic activation or multisensory stimulation also modified inflammatory brain processes via activation of microglia and resulted in clearance of Aβ and p-tau deposition (14), and considerable cognitive benefits (15). Induction of gamma activity in presymptomatic AD mice even prevented subsequent neurodegeneration and behavioral deficits. This suggests that the induction of gamma oscillations may represent a novel and powerful therapeutic approach for AD. A translation of this work to humans seems feasible and promising.

Notably, it has been shown that in humans, transcranial Alternating Current Stimulation (tACS) can safely, and selectively enhance gamma or theta oscillations in specific brain regions (16). Others have also shown that modulation of brain oscillations in humans with other forms of transcranial Electric Stimulation (tES) is possible and may alter mechanisms of brain plasticity (17) and memory functions (18–25). tACS is a safe, non-invasive tES technique that utilizes low-amplitude alternating currents to modulate brain activity and entrain specific cortical rhythms depending on the applied stimulation frequency (26). The effects of tACS modulation of brain oscillations can outlast the duration of stimulation (27). tACS can be targeted to different cortical brain regions depending on the electrode montage. A recent method for optimizing the configuration of multifocal tACS for stimulation of specific brain networks (28, 29) has been developed. Such multifocal brain network targeting achieves a more significant behavioral and physiological impact than traditional tACS approaches using two large electrodes (30).

In a controlled, laboratory-based pilot study, patients with AD underwent 10 or 20 sessions of tACS at the gamma frequency, and brain magnetic resonance imaging (MRI), positron emission tomography (PET) for amyloid, tau or microglial activation, electroencephalography (EEG), and neuropsychological evaluations were completed before and after the intervention. Preliminary results reveal that (1) tACS can induce a significant induction of gamma activity in patients with AD that can be demonstrated on EEG; and (2) tACS-induced modulation of amyloid and tau deposition is demonstrable by PET, consistent with results in the murine models (ClinicalTrials NCT03412604). However, 10 or 20 sessions of stimulation are not enough to induce apparent behavioral and cognitive effects, and long, multi-week courses of laboratory-based interventions are challenging for patients with dementia and their caregivers (unpublished data).

In the present study, we aimed to test the feasibility and safety of a home-based, cost-effective stimulation system that could be used as an intensive neurorehabilitative treatment for long-term memory improvement in patients with dementia. Specifically, our aims were (1) Assess whether repeated, daily sessions of 40 Hz tACS can be applied safely and reliably at home by a caregiver following appropriate instruction; (2) Evaluate the tolerability, acceptability, and adherence of such a home-based program; (3) Gain very preliminary insights as to whether such a stimulation protocol can be run remotely and can lead to memory improvement in older cognitively impaired participants with ADRD. We hypothesized that a multi-week regimen of daily sessions of 40 Hz tACS to the left angular gyrus (AG) would be safe, well-tolerated, and adhered to, and would result in improvements in the memory performance. We selected the AG as the target area, based on previous studies (31–33) and based on the growing evidence that this brain area is critical for the subjective, conscious experience of retrieving vivid, rich and multi-sensory episodic memories (7, 34, 35). Importantly, we hypothesized that this small pilot would establish feasibility

for a large-scale trial conducted at patients' homes with minimal disruption and high scalability.

MATERIALS AND METHODS

Study Participants

ADRD Subjects

The ADRD subjects were both 79 years old men with an established diagnosis of dementia supported by clinical, neuropsychological, neuroimaging, and biomarker results. Both participants were on the same pharmacological regimen throughout the trial, and their medication regimen had been stable for at least 4 months prior to starting the study. Both were taking donepezil at a dose of 5 mg twice per day, and subject #1 was also on sertraline 25 mg once per day. Both participants had a typical amnesic phenotype with no prominent anomia. Both underwent a detailed neurological exam and medical evaluation and a comprehensive neuropsychological battery to characterize cognitive function. Baseline dementia severity was assessed using the Clinical Dementia Rating (CDR) scale (36) and the Montreal Cognitive Assessment (MoCA) (37). The participants' MoCA values using the standard version of the Montreal Cognitive Assessment which includes visual and other tasks, were 21 and 23 at the baseline. Despite the reported overall good correlation between visual, in-person MOCA and the 5 min, telehealth, non-visual MOCA, our two participants showed substantially lower scores in their non-visual MoCA scores (13 and 17, respectively) owing to their particular deficits in verbal memory, verbal word-list generation, and orientation. A cutoff of the MoCA score >18 for inclusion was applied. Both were willing and deemed capable of giving informed consent for participation in the study after the study was thoroughly explained. Understanding of the Informed Consent form was assessed by asking potential participants to answer the following questions: "What is the purpose of the study?", "What are the risks of study involvement?" and "If you decide to participate, are you allowed to withdraw from the study at any time?". Answers were recorded and reviewed for detail and correctness by the study physician. Insufficient understanding or unwillingness to participate would have led to exclusion from the study. Neither participant had any contraindication to tACS, as recorded on a standardized screening questionnaire, which included a history of seizures, use of neuroactive drugs, active dermatological condition (e.g., eczema on the scalp), or presence of specific implanted medical devices (e.g., deep brain stimulator, medication infusion pump, cochlear implant, or pacemaker).

tACS Administrators

The spouses of the two ADRD subjects participated in the study as "tACS administrators" and were taught how to administer home-based tACS. Both administrators self-reported computer proficiency and willingness to learn how to use tACS as defined by "yes" answers to the questions: "Do you feel comfortable using a computer?" and "Are you willing to learn how to administer tACS?". Insufficient understanding or unwillingness to participate would have led to exclusion from the study. The administrators stated their availability during weekdays throughout the study period to administer tACS to the participants. Inclusion criteria included a MoCA score >26 during the in-person screen, sufficient understanding of study procedures following review of the Informed Consent form, and no health condition, such as poor eyesight, that could interfere with the successful administration of the tACS sessions. Both tACS administrators underwent a detailed neurological exam and medical evaluation and a comprehensive neuropsychological battery that ruled out dementia or other significant neurological or psychiatric disorders. Both were female (ages 78 and 73 years), had a CDR of 0, and a MoCA of >28/30 points. None of them had any contra-indication to tACS, as recorded on the same standardized screening questionnaire used in the patients.

EEG Recordings and Analysis

The brain activity of all participants was recorded with high-density EEG during an autobiographical memory task at the beginning of the study. The EEG findings in the two study ADRD participants were compared with those in the two age-matched, cognitive-unimpaired tACS administrators who underwent the same EEG procedure as part of a parallel study protocol approved by the institutional review board.

EEG was recorded with a 256-channel EGI system (Electrical Geodesic Inc., OR, USA), a sampling rate of 1,000 Hz and Cz as acquisition reference. All EEG analysis was performed using the freely available academic software Cartool developed in the Biomedical Imaging Centre Geneva-Lausanne (38) (https://sites.google.com/site/cartoolcommunity/). The EEG was down-sampled offline to 250 Hz; bad electrodes were interpolated using a 3D spherical spline, bandpass filtered between 1 and 100 Hz, and the data were re-referenced to the common average-reference. Infomax-based Independent Component Analysis (ICA) was applied to remove oculomotor and cardiac artifacts.

The EEG was analyzed using time-frequency decomposition to uncover the stimulus-induced synchronization/desynchronization after the presentation of self-relevant pictures and the differences of these spectral changes between controls and the two ADRD subjects. To determine the sources of the stimulus-induced frequency changes, the signal was bandpass filtered in the frequency band of interest. A distributed linear inverse solution (LAURA) (39) was calculated using the average brain of the Montreal Neurological Institute as the head model and restricting the solution space to the gray matter using the LSMAC approach with 5,016 solution points (40). **Figure 1** depicts the roadmap to improve autobiographical memory (**Figure 1A**) using EEG-guided (**Figure 1B**), personal tACS stimulation (**Figure 1C**).

tACS Administration

tACS was delivered by a wireless, battery-driven current stimulator (Starstim SS32, Neuroelectrics), through surface Ag/AgCl electrodes (1 cm^2 radius circular surface contact area) placed into holes of a neoprene cap corresponding to the international 10/20 EEG system, with the Cz position aligned to the vertex of the head. Gel (Signa Gel, Parker Lab, Inc.) was applied to optimize signal conductivity and lower the impedance. Starstim resembles a swimming cap with small electrodes that lie

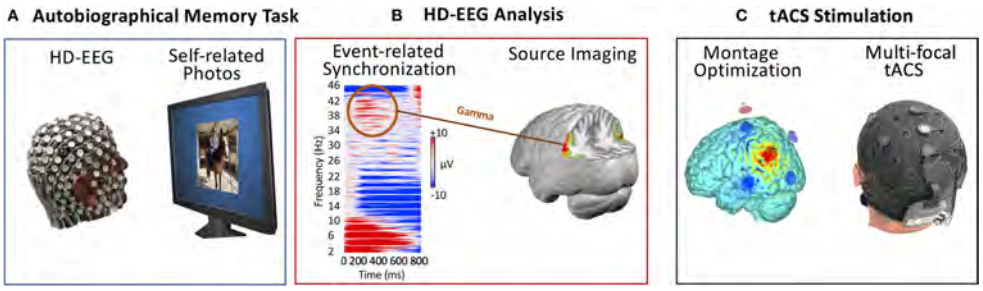

FIGURE 1 | Roadmap of EEG-guided personal tACS to improve autobiographical memory. **(A)** High-density EEG recording during a personalized autobiographical memory task that requests to decide whether pictures are self-related or not. **(B)** Analysis of the high-density EEG in terms of stimulus-evoked frequency modulations. Determination of the individual peak frequency of event-related synchronization and source localization of this frequency based on individual structural MRI head models. **(C)** tACS montage optimization based on the EEG result to perform multi-focal stimulation at the individual gamma peak frequency targeting the sites determined by the EEG source imaging.

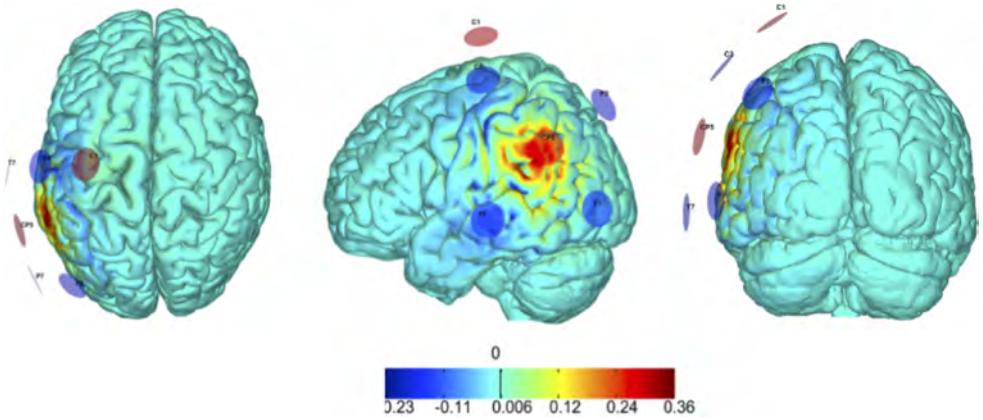

FIGURE 2 | Modeling of electric field induced by tACS. tACS was delivered to the target region (left angular gyrus, BA39/40). Our montage optimization is based on the *StimWeaver* algorithm (29) that revealed that 6 stimulating channels, 2 anodes, and 4 cathodes, provide an optimal montage solution, with the precise stimulation pattern defined using modeling of the induced electric field. By design, the number of active electrodes depended on the optimization but was predefined to be kept always ≤ 8. In keeping with safety guidelines, the max total injected current was set to be < 4.0 mA and a maximum current per electrode was set to be < 2.0 mA.

on the scalp and deliver low-level electrical currents to targeted brain regions. Care was taken to ensure that the caregivers unambiguously placed the color- and number-coded electrodes in the correct position and secured the correct lead to each electrode. The Home Kit included a WIFI-enabled tablet running Starstim control software that provided the caregivers with step-by-step instructions to set up the Starstim device, check electrode impedance, and record side effects. The participants and the caregivers were able to videoconference with the remote staff in real-time. We used Neuroelectrics online Portal (**NE Portal**) to remotely schedule sessions and monitor in real-time specific treatment events to ensure safety.

Feasibility, Safety, and Tolerability

The online NE portal provided information related to electrode impedance, tACS progress if the tACS session was aborted and, if so, then why it was aborted. Following each tACS session, the participants receiving tACS reported side effects and complications via a series of multiple-choice and open-ended questions on the tablet. This information was immediately available to the researcher. These metrics were used to assess feasibility, tolerability, and safety: the number of aborted sessions, the number of missed sessions. The tACS device contains a safety block such that only one tACS session can be administered within 24 h.

tACS Montage Optimization

Based on previous studies (31–33), we selected the left AG as the target area and a stimulation frequency of 40 Hz (gamma range). The AG target area was identified on a standard reference brain, which was also used to determine the optimized montage. In future studies, we suggest the use of a personalized head model for each patient to determine individually optimized montages. Personalized head models can be reconstructed from the individual MRIs of each specific participant, and the target areas should be mapped onto each cortex after registration in common reference space [see, e.g., (41)]. The montage optimization study was performed using the *Stimweaver* algorithm (29), which revealed an optimized solution for the target area (the left AG, BA39/40) with a montage of 6-electrodes in standard EEG electrode positions (circular electrodes with a contact area of 3.14 cm^2). The Stimweaver algorithm does not use fixed assignment of certain electrodes as cathodes and others as anodes based on a pre-established arrangement. This is distinctly different from other multi-electrode approaches like the HD-stim where the central electrode can be the cathode and the surrounding electrodes are the anodes, or vice-versa. Applying this algorithm, we designed montage with 6 active electrodes, 2 anodes and 4 cathodes which induced maximal current in the left AG whilst minimized current induced in the rest of the brain outside the target area. The optimization of the tACS montage was designed to maximize on target the impact of the cortically standard component of the electric field, believed to be the main driver for the effects of tES (29), while avoiding stimulating more extensive off-target areas. The modeling fit quality of the 6-electrode montage was almost 99% of the one allowing for the maximal number of electrodes in the used cap (39 electrodes)—see **Figure 2**. Both participants received the same, well-controlled, and fixed montage (electrode locations and currents). During each session, a maximum total injected current of 4 mA was applied with a maximal current at any one electrode of 2 mA. The current intensity was ramped up over 30 s, then sustained at the stimulation intensity for 20 min, then ramped down over 30 s. A recent study by Reinhart and Nguyen (20) applied single session of tACS for 25 min in older adults and showed an increase in neural synchronization patterns within the frontotemporal brain regions. As a result, they reported a significant improvement in working memory. Therefore, we expected that 20 min would be sufficient to provide a significant effect, but future studies may want to consider longer stimulation sessions. For example, Iaccarino et al. (14) and Martorell et al. (42) applied 1 h stimulation sessions.

Home-Based tACS Training for Administrators

The administrators were trained on how to use the tACS home equipment during a lab-based visit. They received a step-by-step overview of the home-based stimulation kit and completed several training sessions, including tACS administration and equipment care. The administrators then practiced setting up the tACS session on the ADRD participants who received stimulation, with oversight and coaching from the study staff. Study staff initiated a competency checklist (including electrode and machine preparation, stimulation) and identified specific areas that required more focused training. At the end of the training, we assessed and confirmed that the administrators achieved proficiency in applying the home-based tACS against a checklist of procedures confirming the ability to prepare the equipment independently and to operate the device safely. Administrators demonstrated competency in all procedures and self-reported comfort in doing so.

A pre-configured tACS system, including the device, tablet, all needed supplies, and training material was provided to the each of the ADRD participant-administrator pair. The tACS tablet contained a sequence of simplified instructions, and step-by-step touchscreen prompts for the participant/administrator to follow. This process was designed to be "older-adult-friendly" and easy to use, even if the administrators were not advanced computer users. A companion paper-based manual was delivered to the participants together with the tablet instructions. The trained administrators then applied 20 min-long tACS sessions in the morning to the participants at home for 14 weeks, 5 days per week, for a total of 70 sessions per participant. During the tACS sessions, participants were seated comfortably and remained at rest, stayed awake, and refrained from talking. The administrators were instructed to observe the participants, and if necessary, to encourage them to stay awake during each tACS session. In the beginning and at the end of each tACS session, participants completed a questionnaire to report potential side effects (e.g., skin irritation, headache, changes in alertness etc.).

Cognitive Evaluation of the Participants

Since the evaluations were done remotely, we used the Mini/5 min, non-visual version of the MoCA (37, 43) to assess the cognitive effects of the intervention bi-weekly. Three different versions of the MoCA were employed to minimize the risk of carry-over and learning effects. The non-visual MoCA scores of the two ADRD subjects were 13 and 17 in the beginning of the study and 29 and 21 at the end of 14 weeks of home-based tACS stimulation. Given the focus and aims of the study, we focused on the Memory Index Score as defined and adopted in the Uniform data set of the National Alzheimer's Coordinating Center. The Memory Index Score is based on the delayed recall of 5 words and awards 3 points for each word that is freely recalled without a cue for a maximal score of 15. Thereafter, cues were offered only for words not recalled spontaneously. Two points were awarded for each word recalled with a categorical cue (e.g., "one of the words was a color"). Finally, 1 point was awarded for each of the still missing words correctly identified on recognition (e.g., "was one of the words daisy, rose, or tulip"). Both participants were tested every 4 weeks after the completion of the study for potential carryover effects.

RESULTS

Our preliminary results document the feasibility of the approach, support the safety of the intervention, and provide essential

information regarding the potential beneficial effects of tACS on cognitive function in ADRD participants.

EEG Results

The results of the high-density (hd) EEG data using time-frequency analysis (**Figure 3**) are consistent with the literature: the memory task-induced frequency power increases in the theta (2–8 Hz) and the gamma range (36–42 Hz) and power decrease in the alpha and beta range (10–30 Hz) (**Figure 3A**). No stimulus-induced changes were observed in the high gamma range. The memory stimuli-induced increases in theta and gamma oscillations were reduced in the two AD patients as compared to nine cognitively-unimpaired controls (6 females, age range 66 ± 8 years) (**Figure 3B**). Source localization of the gamma oscillations (36–42 Hz bandpass filtered EEG) revealed generators in the inferior parietal lobule (Brodmann Area 39) (**Figure 3C**).

Adherence, Safety Monitoring, and Risk Assessments

Both participant-administrator pairs showed perfect adherence to the protocol (100%), missing none of the 70 scheduled sessions in the 14 study weeks. The researchers did not have to intervene to resolve any major technical difficulties. In total, the sessions were twice auto-aborted due to high impedance. It is noteworthy that the lab-based training, including an assessment of competency which the administrators needed to pass prior to starting the study, prepared the administrators for such situations and therefore they were able to independently complete the stimulation sessions. The home-based stimulation sessions were remotely supervised by the researchers who received information about impedance, tACS progress, and, potentially, session aborts or interruptions in real-time. The participants and administrators could initiate a video conference before, during, or after the stimulation sessions and immediately communicate or report any issue, if necessary, to the researchers. The study was designed to minimize risks to the study participants. The tACS was ramped up and down to reduce the occurrence of unpleasant sensations under the electrode. The participants were monitored throughout the stimulation period and were evaluated by a researcher each day before and after the stimulation. All home stimulation sessions were, therefore, supervised.

Report on Tolerability, Side Effects, and Technical Difficulties

Participants completed self-report measures to address tolerability before and after each session. Reported side effects were the expected (44), common side-effects of tACS and were all mild. All the side effects resolved after the completion of the stimulation session. Side effects mainly included sensations of tingling and burning on the electrode site. There were no skin lesions. There were few occurrences of headache, and one occurrence of difficulty concentrating following a session. Reported technical difficulties during the home-based sessions were common of tACS and mild. Technical issues mainly included measures of high electrode contact impedance, which resolved after adding extra gel under the specific electrodes. There were few occurrences of session interruptions and aborts due to high-impendence and due to internet connection cutoffs. In all instances, it was possible to address the issue and resume the session with minimal delay. None of them led to the missed sessions (all sessions were completed).

Cognitive Effects

Performance in Formal Testing

Over the 14 weeks of the study, both participants exhibited an improvement in the testing completed every 2 weeks as compared with baseline (**Figure 4**). These data are uncontrolled and on only two participants and should, therefore, be considered extremely preliminary. Nonetheless, the study provides clear, initial evidence that home-based, remotely supervised tACS treatment is feasible for participants with memory deficits and offers a potentially effective intervention.

In the Memory Index assessment, at baseline, one of the participants (**Figure 4**, red) was not able to recall any of the words, not even after categorical or multiple-choice cues, and in fact, did not also recall having been given any words to remember. At the end of the 14 weeks, however, he correctly freely recalled all 5 words. The other participant at baseline was only able to identify three of the five words from the multiple-choice cue, but at the end of the tACS course, he freely recalled three of the words, recalled the fourth with a categorical cue and correctly identified the last one from multiple-choice options. Of note, that participant had obstructive sleep apnea, and midway through the tACS course had his CPAP device exchanged because of concerns that it was not working correctly. Following the exchange of the CPAP device, the participant reported feeling more rested in the morning and having a "clearer head," and the Memory Index score improved substantially (from 5 to 11).

Qualitative Report of the Impact of Daily Activities

Both participant pairs were queried bi-weekly on their qualitative impressions regarding cognitive and memory performance. The spouses reported noticing definite improvements in the daily activities of their husbands but also commented on fluctuations from day to day. Both commented, though, on increasing number of instances where they were surprised, their husbands recalled a given piece of information or event. Overall they both felt there were fewer instances of repeating asking of the same questions, better orientation, greater recall of plans, etc. For example, one of the participants and his wife play bridge, and the wife commented that the ADRD participant's play improved substantially, with his being able to follow the games better, recall conventions, and overall regaining a level of play he seemed to have lost. At another time, the wife noted that he recalled not only playing golf the day before but also with whom and what the scores had been.

DISCUSSION

This study provides preliminary data to support the feasibility, safety, and home-based tACS in the ADRD population and encourages future research to increase access of tACS as part of memory decline prevention strategies and treatment for older adults with memory problems. Our data demonstrate that

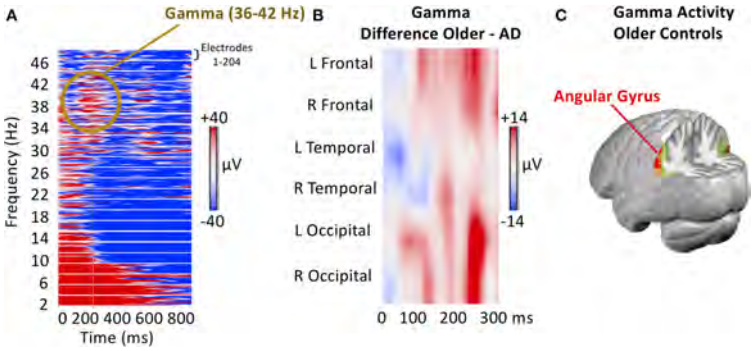

FIGURE 3 | EEG Frequency modulation induced by the presentation of autobiographical photographs. **(A)** Example of induced power changes from 2 to 44 Hz after subjects responded, indicating having recognized a picture as self-related. Each line represents the 257 electrodes in the given frequency. Note the power increase in theta and gamma and the decrease in alpha. **(B)** The induced gamma power difference between participants with ADRD and age-matched cognitively-unimpaired controls. Electrodes are averaged for regions of interest, as indicated. Note the more considerable gamma power increase for recognized pictures in controls as compared to ADRD participants. **(C)** Source localization of task-related gamma activity increase averaged across the period of 100–300 ms after stimulus onset. Note the left inferior parietal activity in the region of the angular gyrus.

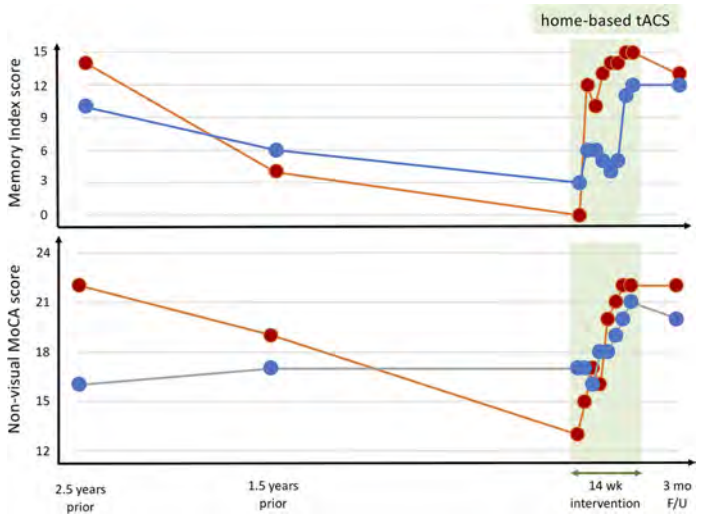

FIGURE 4 | Cognitive performance throughout a 14-week intervention of open-label, home-based, caregiver-administered tACS in two ADRD participants. Both participants completed all scheduled stimulation sessions and tolerated the intervention with no report of adverse events or complications. The Mini/5 min non-visual versions of the Montreal Cognitive Assessment (MOCA) were evaluated via telehealth rotating between three different versions to avoid learning or carry-over effects. The equivalent metrics from the standard MOCA were extracted from the neuropsychological evaluations completed on both participants 2.5 and 1.5 years before study participation. By definition, the control subjects had normal memory index scores (a score of 14 or 15 in all cases) and normal MOCA scores (a score of 27–30 in all cases).

caregivers can be trained to apply home-based tACS competently and that tele-monitored tACS in participants with ADRD is well-tolerated and safe even in repeated consecutive daily sessions over many weeks. Furthermore, even though the data have to be taken with great caution given the uncontrolled nature of the intervention and the very small N, our data suggest that in

participants with ADRD, tACS can induce clinically meaningful cognitive effects.

The EEG and the tACS results of this pilot study suggest that the left angular gyrus may be a suitable target brain area of the memory network for spatially-specific modulation of brain oscillations to induce memory enhancement. The angular gyrus has been recently shown to be involved in episodic, self-relevant memories (31–33), which is one of the earliest AD memory deficits. Patients with posterior parietal damage exhibit impaired free recall of autobiographical memories, however when cued, their memories seem to be intact (45). Even if the memory accuracy appears to be unaffected by the parietal lesions, the vividness, richness and subjective confidence in the personal memories is diminished (46, 47). Due to its specific cognitive functionality, we chose the left AG as the target region for brain stimulation. The targeted area is strongly associated with the internally directed processes, such as mind-wandering or thinking about one's past and future. The AG has been shown to activate during retrieval of episodic autobiographical memories. Imaging studies on mind-wandering have consistently shown activation in angular gyrus associated with the DMN. Interestingly, the disruption of the DMN in AD has been reported (48, 49). Our results thus have profound translational potential as tACS intervention is safe, portable, does not require expensive hardware, and can be widely applied to large numbers of MCI/ADRD participants as well as adults at earlier stages of other, related neurodegenerative diseases.

Histopathologically, AD is characterized by diffuse amyloid-β (Aβ) plaques and phosphorylated tau (p-tau) deposition in neurofibrillary tangles, as well as widespread neurodegeneration. PET imaging studies suggest that progressive Aβ deposition can begin decades before the onset of clinical symptoms and stabilizes around the time that clinical symptoms become prominent (50, 51). P-tau accumulates initially in the meso-temporal lobes and spreads outside the temporal lobes linked with Aβ. Neurodegeneration and clinical symptoms seem strongly correlated with the spread of p-tau (52). Memory network disfunction is an early, primary driver of cognitive disability and disease progression in dementia (53, 54). Recent studies demonstrate that in AD, even before the accumulation of Aβ and p-tau, Aβ oligomers cause synapse-specific dysfunctions in parvalbumin-positive (PV+) and somatostatin-positive (SST+) interneurons leading to a dysregulation of theta and gamma oscillations. Loss of gamma activity has been linked to alterations in the mechanisms of brain plasticity (55–57), an early finding in AD also thought to be related to the synaptic toxicity of Aβ oligomers (58, 59). Disrupted theta and gamma oscillatory activity is reflective of altered functional and structural brain integrity (60) and heralds p-tau and Aβ accumulation, memory deficits, and cognitive decline in AD (61).

Recent lab-based studies suggest the feasibility of interacting with brain oscillations using tACS. Due to its safety (44) and controllability (in terms of stimulation frequency and the possibility to target specific cortical regions), tACS appears to be a most promising technique to modulate activity in the healthy and pathological brain. Animal work has demonstrated that tACS can entrain neurons in widespread cortical areas (62) with the effects constrained by the individual endogenous cortical oscillations (26, 63–65). Simulations (26, 64, 66), supported by empirical evidence using EEG, demonstrate that tACS modulates oscillatory brain activity via network resonance, suggesting that a weak stimulation at a resonant frequency can cause large-scale modulation of network activity (67), and amplify endogenous network oscillations in a frequency-specific manner (26). In humans, tACS modulates brain activity, with effects being documented at the behavioral level for sensorimotor (68, 69), visual (70), somatosensory (71) and higher-order cognitive domains (19, 72), and lasting beyond the duration of stimulation (27). However, tACS interventions despite their safety are currently not accessible to large numbers of older adults who live in remote settings or who are unable to utilize and organize transportation, because such interventions must be administered over multiple in-person visits to a clinical or laboratory setting. This barrier hinders the ability of healthcare professionals to deliver tACS to those who stand to benefit the most. It also places a sizeable travel-related burden on clinical trials and thus slows the pace of discovery and technology transfer of tACS to vulnerable older adult populations. Our study addresses this need by demonstrating the feasibility of a remotely supervised and remotely controlled tACS in memory-related disorders and home-based settings.

Our primary goal was to demonstrate the feasibility of remotely-monitored, caregiver (or spouse)-administered, home-based tACS intervention to improve memory in older adults by modifying gamma activity. To do so, we used the Starstim Home Kit® (Neuroelectrics Corp). The Starstim Home Kit enabled us to "prescribe" home-based tACS to end-users. Following in-person training sessions in the lab, two ADRD participants and their caregivers took home a pre-configured and personalized Starstim system. The researcher monitored treatment progress remotely in real-time. Our preliminary findings reveal the promising nature of tACS as a paradigm for participants where repeated visits to the clinic are not feasible and provide the first evidence of adherence to multi-week, home-based tACS memory treatment. It is noteworthy that the current protocol is established in such a way that after the initial lab-based training, the healthy caregiver is able to run the home-based sessions independently and without continuous contact with the researcher. Participation from home, while still maintaining clinical trial standards and high-quality research, can resolve accessibility barriers and improve recruitment, thus enabling the implementation of more substantial and longer-lasting clinical trials. Our approach opens a new avenue for several innovative methodological features, including (1) a personalized multi-electrode montage will enable modulation of specific brain regions (29) (2) advanced modeling will provide insights into the tACS dose induced in the targeted brain areas (73).

Limitations and Future Directions

We limited the EEG analysis on event-related spectral perturbation. Potentially interesting analysis would be of the ratio between high and low frequencies, as 1/f noise has been shown to increase with age (74). This 1/f analysis and

its relation to clinical outcomes would be ideal to include in future studies. Future studies should also explore how the effects of tACS affect verbal vs. non-verbal memory, auditory procession, attention or lexical retrieval. This could be done by including diverse tests, specific for each category. Even though the clinical evaluation was performed every 2 weeks during the 14 weeks of the study and then every 4 weeks after the completion of the study, we cannot exclude a learning effect of the actual task. Therefore, future studies should include a diversity of cognitive tasks and evaluations. Given our design, we cannot be certain that the effects of daily home-based stimulation are specific to the gamma frequency. Therefore, future studies should consider appropriate controls for example in a parallel or cross-over trial design, which might ideally include sham stimulation sessions as well as tACS at other stimulation frequencies. Along the same lines, including a control condition of the target area would be important to evaluate whether targeting the angular gyrus is indeed critical. Future studies should also examine and thoroughly test the effects of stimulation based on participant's circadian rhythm. We found a carryover effect lasting up to 3 months after the end of the study. Future studies should examine further the longevity of such effects. Finally, it would be profitable for future studies to focus on biomarker+ AD (either positive amyloid PET scans or CSF) and gather structural and functional MRI scans at the beginning of the study and compare such markers at the end of the study.

Multi-Electrode tACS for Precise Targeting

The spatial resolution of transcranial Electric Stimulation (tES) in general, and tACS, in particular, is limited. However, new technologies to increase the precision of tES targeting have been recently developed, allowing for individualized stimulation solutions based on modeling of current distribution using brain MRI scans. Commonly used tES devices only allow for stimulation protocols using two electrodes, limiting control of the target regions. Moreover, this does not allow for careful mapping of individual brain anatomy, which might be even more critical in the case of individuals with AD, where significant levels of cortical atrophy are expected, which might dramatically affect the induced electrical field. Multi-focal (i.e., multi-electrode) stimulation (29) permits montages with up to 32 stimulating channels, with the stimulation pattern defined using modeling of the induced electric field based on MRI scans. This approach allows for individualized montages to target specific brain networks. Given the cortical atrophy expected in patients with AD, individual tES models based on structural MRI will be necessary also to account for increased scalp-cortex distance and CSF volume, which can significantly alter the distribution of the induced electric field (75–77). Furthermore, current research efforts are being directed not only to more realistic modeling of the physics of tES and its associated electrical field but to its physiological impact. Such *hybrid brain models* integrate physics and physiology using neural mass models assembled with structural and physiological data (MRI, DTI, EEG). This framework will allow for more realistic and informed tES targeting and optimization strategies (78).

Home-Based, Remote Technology-Supported Cognitive Assessment

The recent shut-down due to the world-wide pandemic has challenged the traditional model of running research studies in a laboratory environment. Since it seems feasible and safe to monitor a caregiver to administer home-based tACS interventions remotely, our results emphasize the need for reliable, validated remote, technology-supported cognitive assessment and digital measurements. We completed the present study conducting the MoCA using telehealth. However, more sensitive, remote technology-supported evaluations are needed. Critically, most of the neuropsychological assessments of memory are often disconnected from the personal, self-related events that AD participants experience in everyday life. Therefore, testing autobiographical memory in a more naturalistic, ecological way rather than using laboratory-based tasks, which are mostly self-irrelevant, would be beneficial for the AD participants. The traditional Clock Drawing Test (79) is a well-established and widely used pen and paper cognitive assessment in which participants draw the face of a clock showing a specific time on a blank page, then copy a pre-drawn clock. However, the DCTclock^{TM} online test uses new advances in technology to capture and analyze the small movements of drawing behavior remotely (80, 81) and provides more sensitive metrics that are correlated with tau and amyloid loads on PET in patients with AD (82). Such methods of evaluation would be ideal to combine with the home-based tACS sessions. Similarly, E-Prime go (https://pstnet.com/using-e-prime-for-remote-data-collection/) is a psychology software tool that enables to send experiments to participants at their homes and thus collect data remotely. The MoCA assessment itself is available as an online app and has been recently validated (83, 84). BrainCheck (https://braincheck.com) and others offer suitable, FDA-cleared computer-based cognitive assessments that can be acquired remotely. Linus Health (https://linus.health) captured multimodal signals, not only performance on neuropsychological tasks (including the DCTclock^{TM}) but also voice, eye movement, and gait and balance metrics to provide a more holistic and ecologically valid measure of cognitive and behavioral function. These online platforms for remote data collection and assessment seem to be extremely promising.

Conclusion

To our knowledge, this is the first remotely supervised tACS protocol designed explicitly for ADRD participants. The results of our exploratory study encourage more extensive, randomized sham-controlled studies of the therapeutic potential of gamma tACS over the left angular gyrus in ADRD. The possibility of effectively delivering tACS neuromodulation in home-based environments in a standardized, supervised fashion opens

stimulating perspectives for the definition of new paradigms of neurorehabilitation and the development of well-powered, well-designed clinical trials.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors to qualified parties upon written request and consideration.

ETHICS STATEMENT

The study was approved by the Institutional Review Board at Hebrew SeniorLife and conducted according to principles outlined by the Declaration of Helsinki and the International Ethical Guidelines for Biomedical Research Involving Human Subjects. Two pairs of participants, a subject with ADRD and prominent memory deficits and a caregiver capable and willing to learn and then apply the home-based tACS sessions, were recruited into this pilot study by the research study team following appropriate screening, which included detailed description of the study protocol, tACS eligibility screening, computer proficiency screening (see more details in section study participants), and written informed consent. These two pairs were the first two recruited into an ongoing study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LB, WY, MB, GR, MG, BM, and AP-L: conceptualization. LB, WY, MB, GR, and AP-L: methodology. MB, GR, and AP-L: software. LB, MB, GR, and AP-L: formal analysis and visualization. LB and AP-L: data curation and writing—original draft preparation. LB, MB, GR, BM, and AP-L: writing—review and editing. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported primarily by a generous philanthropic contribution and the Swiss National Science Foundation through the SNSF Early Post-Doc Mobility grant (187949) to LB. AP-L was partly supported by the National Institutes of Health (R24AG06142 and P01 AG031720). BM was supported by R01 AG059089-01.

REFERENCES

1. Levine B, Turner GR, Tisserand D, Hevenor SJ, Graham SJ, McIntosh AR. The functional neuroanatomy of episodic and semantic autobiographical remembering: a prospective functional MRI study. *J Cogn Neurosci.* (2004) 16:1633–46. doi: 10.1162/0898929042568587
2. Schacter DL, Addis DR, Hassabis D, Martin VC, Spreng RN, Szpunar KK. The future of memory: remembering, imagining, the brain. *Neuron.* (2012) 76:677–94. doi: 10.1016/j.neuron.2012.11.001
3. Svoboda E, McKinnon MC, Levine B. The functional neuroanatomy of autobiographical memory: a meta-analysis. *Neuropsychologia.* (2006) 44:2189–208. doi: 10.1016/j.neuropsychologia.2006.05.023
4. Scoville WB, Milner B. Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry.* (1957) 20:11–21. doi: 10.1136/jnnp.20.1.11
5. Steinvorth S, Levine B, Corkin S. Medial temporal lobe structures are needed to re-experience remote autobiographical memories: evidence from H.M. and W. R. *Neuropsychologia.* (2005) 43:479–96. doi: 10.1016/j.neuropsychologia.2005.01.001
6. Cabeza R, St Jacques P. Functional neuroimaging of autobiographical memory. *Trends Cogn Sci.* (2007) 11:219–27. doi: 10.1016/j.tics.2007.02.005
7. Ciaramelli E, Faggi G, Scarpazza C, Mattioli F, Spaniol J, Ghetti S, et al. Subjective recollection independent from multifeatural context retrieval following damage to the posterior parietal cortex. *Cortex.* (2017) 91:114–25. doi: 10.1016/j.cortex.2017.03.015
8. Buzsaki G. Theta oscillations in the hippocampus. *Neuron.* (2002) 33:325–40. doi: 10.1016/S0896-6273(02)00586-X
9. Buzsaki G, Moser EI. Memory, navigation and theta rhythm in the hippocampal-entorhinal system. *Nat Neurosci.* (2013) 16:130–8. doi: 10.1038/nn.3304
10. Fell J, Axmacher N. The role of phase synchronization in memory processes. *Nat Rev Neurosci.* (2011) 12:105–18. doi: 10.1038/nrn2979
11. Babiloni C, Lizio R, Marzano N, Capotosto P, Soricelli A, Triggiani AI, et al. Brain neural synchronization and functional coupling in Alzheimer's disease as revealed by resting state EEG rhythms. *Int J Psychophysiol.* (2016) 103:88–102. doi: 10.1016/j.ijpsycho.2015.02.008
12. Verret L, Mann EO, Hang GB, Barth AM, Cobos I, Ho K, et al. Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell.* (2012) 149:708–21. doi: 10.1016/j.cell.2012.02.046
13. Nyhus E, Curran T. Functional role of gamma and theta oscillations in episodic memory. *Neurosci Biobehav Rev.* (2010) 34:1023–35. doi: 10.1016/j.neubiorev.2009.12.014
14. Iaccarino HF, Singer AC, Martorell AJ, Rudenko A, Gao F, Gillingham TZ, et al. Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature.* (2016) 540:230–5. doi: 10.1038/nature20587
15. Adaikkan C, Tsai LH. Gamma entrainment: impact on neurocircuits, Glia, therapeutic opportunities. *Trends Neurosci.* (2020) 43:24–41. doi: 10.1016/j.tins.2019.11.001
16. Santarnecchi E, Sprugnoli G, Bricolo E, Costantini G, Liew SL, Musaeus CS, et al. Gamma tACS over the temporal lobe increases the occurrence of Eureka! moments. *Sci Rep.* (2019) 9:5778. doi: 10.1038/s41598-019-42192-z
17. Guerra A, Suppa A, Bologna M, D'Onofrio V, Bianchini E, Brown P, et al. Boosting the LTP-like plasticity effect of intermittent theta-burst stimulation using gamma transcranial alternating current stimulation. *Brain Stimul.* (2018) 11:734–42. doi: 10.1016/j.brs.2018.03.015
18. Hanslmayr S, Axmacher N, Inman CS. Modulating human memory via entrainment of brain oscillations. *Trends Neurosci.* (2019) 42:485–99. doi: 10.1016/j.tins.2019.04.004
19. Polania R, Nitsche MA, Korman C, Batsikadze G, Paulus W. The importance of timing in segregated theta phase-coupling for cognitive performance. *Curr Biol.* (2012) 22:1314–8. doi: 10.1016/j.cub.2012.05.021
20. Reinhart MG, Nguyen JA. Working memory revived in older adults by synchronizing rhythmic brain circuits. *Nat Neurosci.* (2019) 22:820–7. doi: 10.1038/s41593-019-0371-x
21. Roberts BM, Clarke A, Addante RJ, Ranganath C. Entrainment enhances theta oscillations and improves episodic memory. *Cogn Neurosci.* (2018) 9:181–93. doi: 10.1080/17588928.2018.1521386
22. Violante IR, Li LM, Carmichael DW, Lorenz R, Leech R, Hampshire A, et al. Externally induced frontoparietal synchronization modulates network dynamics and enhances working memory performance. *Elife.* (2017) 6:e22001. doi: 10.7554/eLife.22001

23. Vosskuhl J, Huster RJ, Herrmann CS. Increase in short-term memory capacity induced by down-regulating individual theta frequency via transcranial alternating current stimulation. *Front Hum Neurosci.* (2015) 9:257. doi: 10.3389/fnhum.2015.00257
24. Wang JX, Rogers LM, Gross EZ, Ryals AJ, Dokucu ME, Brandstatt KL, et al. Targeted enhancement of cortical-hippocampal brain networks and associative memory. *Science.* (2014) 345:1054–7. doi: 10.1126/science.1252900
25. Wolinski N, Cooper NR, Sauseng P, Romei V. The speed of parietal theta frequency drives visuospatial working memory capacity. *PLoS Biol.* (2018) 16:e2005348. doi: 10.1371/journal.pbio.2005348
26. Frohlich F, McCormick DA. Endogenous electric fields may guide neocortical network activity. *Neuron.* (2010) 67:129–43. doi: 10.1016/j.neuron.2010.06.005
27. Kasten FH, Dowsett J, Herrmann CS. Sustained aftereffect of alpha-tACS lasts up to 70 min after stimulation. *Front Hum Neurosci.* (2016) 10:245. doi: 10.3389/fnhum.2016.00245
28. Mencarelli L, Menardi A, Neri F, Monti L, Ruffini G, Salvador R, et al. Impact of network-targeted multichannel transcranial direct current stimulation on intrinsic and network-to-network functional connectivity. *J Neurosci Res.* (2020) 98:1843–56. doi: 10.1002/jnr.24690
29. Ruffini G, Fox MD, Ripolles O, Miranda PC, Pascual-Leone A. Optimization of multifocal transcranial current stimulation for weighted cortical pattern targeting from realistic modeling of electric fields. *Neuroimage.* (2014) 89:216–25. doi: 10.1016/j.neuroimage.2013.12.002
30. Fischer DB, Fried PJ, Ruffini G, Ripolles O, Salvador R, Banus J, et al. Multifocal tDCS targeting the resting state motor network increases cortical excitability beyond traditional tDCS targeting unilateral motor cortex. *Neuroimage.* (2017) 157:34–44. doi: 10.1016/j.neuroimage.2017.05.060
31. Brechet L, Brunet D, Birot G, Gruetter R, Michel CM, Jorge J. Capturing the spatiotemporal dynamics of self-generated, task-initiated thoughts with EEG and fMRI. *Neuroimage.* (2019) 194:82–92. doi: 10.1016/j.neuroimage.2019.03.029
32. Brechet L, Grivaz P, Gauthier B, Blanke O. Common recruitment of angular gyrus in episodic autobiographical memory and bodily self-consciousness. *Front Behav Neurosci.* (2018) 12:270. doi: 10.3389/fnbeh.2018.00270
33. Thakral PP, Madore KP, Schacter DL. A role for the left angular gyrus in episodic simulation and memory. *J Neurosci.* (2017) 37:8142–49. doi: 10.1523/JNEUROSCI.1319-17.2017
34. Berryhill ME. Insights from neuropsychology: pinpointing the role of the posterior parietal cortex in episodic and working memory. *Front Integr Neurosci.* (2012) 6:31. doi: 10.3389/fnint.2012.00031
35. Vilberg KL, Rugg MD. Temporal dissociations within the core recollection network. *Cogn Neurosci.* (2014) 5:77–84. doi: 10.1080/17588928.2013.860088
36. Morris JC. The clinical dementia rating (CDR): current version and scoring rules. *Neurology.* (1993) 43:2412–4. doi: 10.1212/WNL.43.11.2412-a
37. Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, et al. The montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc.* (2005) 53:695–9. doi: 10.1111/j.1532-5415.2005.53221.x
38. Brunet D, Murray MM, Michel CM. Spatiotemporal analysis of multichannel EEG: CARTOOL. *Comput Intell Neurosci.* (2011) 2011:813870. doi: 10.1155/2011/813870
39. Grave de Peralta Menendez R, Murray MM, Michel CM, Martuzzi R, Gonzalez Andino SL. Electrical neuroimaging based on biophysical constraints. *Neuroimage.* (2004) 21:527–39. doi: 10.1016/j.neuroimage.2003.09.051
40. Michel CM, Brunet D. EEG source imaging: a practical review of the analysis steps. *Front Neurol.* (2019) 10:325. doi: 10.3389/fneur.2019.00325
41. Miranda PC, Callejón-Leblic MA, Salvador R, Ruffini G. Realistic modeling of transcranial current stimulation: the electric field in the brain. *Curr Opin Biomed Eng.* (2018) 8:20–7. doi: 10.1016/j.cobme.2018.09.002
42. Martorell AJ, Paulson AL, Suk HJ, Abdurrob F, Drummond GT, Guan W, et al. Multi-sensory gamma stimulation ameliorates Alzheimer's-associated pathology and improves cognition. *Cell.* (2019) 177:256–71.e22. doi: 10.1016/j.cell.2019.02.014
43. Wong A, Nyenhuis D, Black SE, Law LS, Lo ES, Kwan PW, et al. Montreal cognitive assessment 5-minute protocol is a brief, valid, reliable, and feasible cognitive screen for telephone administration. *Stroke.* (2015) 46:1059–64. doi: 10.1161/STROKEAHA.114.007253
44. Antal A, Alekseichuk I, Bikson M, Brockmoller J, Brunoni AR, Chen R, et al. Low intensity transcranial electric stimulation: safety, ethical, legal regulatory and application guidelines. *Clin Neurophysiol.* (2017) 128:1774–809. doi: 10.1016/j.clinph.2017.06.001
45. Berryhill ME, Phuong L, Picasso L, Cabeza R, Olson IR. Parietal lobe and episodic memory: bilateral damage causes impaired free recall of autobiographical memory. *J. Neurosci.* (2007) 27, 14415–23. doi: 10.1523/JNEUROSCI.4163-07.2007
46. Hower KH, Wixted J, Berryhill ME, Olson IR. Impaired perception of mnemonic oldness, but not mnemonic newness, after parietal lobe damage. *Neuropsychologia.* (2014) 56:409–17. doi: 10.1016/j.neuropsychologia.2014.02.014
47. Simons JS, Peers PV, Mazuz YS, Berryhill ME, Olson IR. Dissociation between memory accuracy and memory confidence following bilateral parietal lesions. *Cereb Cortex.* (2010) 20:479–85. doi: 10.1093/cercor/bhp116
48. Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T, et al. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *J Neurosci.* (2009) 29:1860–73. doi: 10.1523/JNEUROSCI.5062-08.2009
49. Ferguson MA, Lim C, Cooke D, Darby RR, Wu O, Rost NS, et al. A human memory circuit derived from brain lesions causing amnesia. *Nat Commun.* (2019) 10:3497. doi: 10.1038/s41467-019-11353-z
50. Jack CRJr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* (2010) 9:119–28. doi: 10.1016/S1474-4422(09)70299-6
51. Jack CRJr, Wiste HJ, Lesnick TG, Weigand SD, Knopman DS, et Vemuri P, al. Brain beta-amyloid load approaches a plateau. *Neurology.* (2013) 80:890–6. doi: 10.1212/WNL.0b013e3182840bbe
52. Pontecorvo MJ, Devous MDSr, Navitsky M, Lu M, Salloway S, Schaerf FW, et al. Relationships between flortaucipir PET tau binding and amyloid burden, clinical diagnosis, age and cognition. *Brain.* (2017) 140:748–63. doi: 10.1093/brain/aww334
53. Palop JJ, Mucke L. Network abnormalities and interneuron dysfunction in Alzheimer disease. *Nat Rev Neurosci.* (2016) 17:777–92. doi: 10.1038/nrn.2016.141
54. Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, et al. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. *Proc Natl Acad Sci USA.* (2012) 109:E2895–903. doi: 10.1073/pnas.1121081109
55. Bocchio M, Nabavi S, Capogna M. Synaptic plasticity, engrams, and network oscillations in amygdala circuits for storage and retrieval of emotional memories. *Neuron.* (2017) 94:731–43. doi: 10.1016/j.neuron.2017.03.022
56. Buzsaki G, Wang XJ. Mechanisms of gamma oscillations. *Annu Rev Neurosci.* (2012) 35:203–25. doi: 10.1146/annurev-neuro-062111-150444
57. Mably AJ, Colgin LL. Gamma oscillations in cognitive disorders. *Curr Opin Neurobiol.* (2018) 52:182–7. doi: 10.1016/j.conb.2018.07.009
58. Benarroch EE. Glutamatergic synaptic plasticity and dysfunction in Alzheimer disease: emerging mechanisms. *Neurology.* (2018) 91:125–32. doi: 10.1212/WNL.0000000000005807
59. Pozueta J, Lefort R, Shelanski ML. Synaptic changes in Alzheimer's disease and its models. *Neuroscience.* (2013) 251:51–65. doi: 10.1016/j.neuroscience.2012.05.050
60. Sander MC, Fandakova Y, Grandy TH, Shing YL, Werkle-Bergner M. Oscillatory mechanisms of successful memory formation in younger and older adults are related to structural integrity. *Cereb Cortex.* (2020) 30:3744–58. doi: 10.1093/cercor/bhz339
61. Ranasinghe KG, Cha J, Iaccarino L, Hinkley LB, Beagle AJ, Pham J, et al. Neurophysiological signatures in Alzheimer's disease are distinctly associated with TAU, amyloid-beta accumulation, cognitive decline. *Sci Transl Med.* (2020) 12:eaaz4069. doi: 10.1126/scitranslmed.aaz4069
62. Ozen S, Sirota A, Belluscio MA, Anastassiou CA, Stark E, Koch C, et al. Transcranial electric stimulation entrains cortical neuronal populations in rats. *J Neurosci.* (2010) 30:11476–85. doi: 10.1523/JNEUROSCI.5252-09.2010
63. Marquez-Ruiz J, Ammann C, Leal-Campanario R, Ruffini G, Gruart A, Delgado-Garcia JM. Synthetic tactile perception induced by transcranial

alternating-current stimulation can substitute for natural sensory stimulus in behaving rabbits. *Sci Rep*. (2016) 6:19753. doi: 10.1038/srep19753
64. Santarnecchi E, Brem AK, Levenbaum E, Thompson T, Kadosh RC, Pascual-Leone A. Enhancing cognition using transcranial electric stimulation. *Curr Opin Behav Sci*. (2015) 4:171–8. doi: 10.1016/j.cobeha.2015.06.003
65. Santarnecchi E, Rossi S. Advances in the neuroscience of intelligence: from brain connectivity to brain perturbation. *Span J Psychol*. (2016) 19:E94. doi: 10.1017/sjp.2016.89
66. Merlet I, Birot G, Salvador R, Molaee-Ardekani B, Mekonnen A, Soria-Frish A, et al. From oscillatory transcranial current stimulation to scalp EEG changes: a biophysical and physiological modeling study. *PLoS ONE*. (2013) 8:e57330. doi: 10.1371/journal.pone.0057330
67. Schmidt SL, Iyengar AK, Foulser AA, Boyle MR, Frohlich F. Endogenous cortical oscillations constrain neuromodulation by weak electric fields. *Brain Stimul*. (2014) 7:878–89. doi: 10.1016/j.brs.2014.07.033
68. Feurra M, Bianco G, Santarnecchi E, Del Testa M, Rossi A, Rossi S. Frequency-dependent tuning of the human motor system induced by transcranial oscillatory potentials. *J Neurosci*. (2011) 31:12165–70. doi: 10.1523/JNEUROSCI.0978-11.2011
69. Feurra M, Pasqualetti P, Bianco G, Santarnecchi E, Rossi A, Rossi S. State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. *J Neurosci*. (2013) 33:17483–9. doi: 10.1523/JNEUROSCI.1414-13.2013
70. Kanai R, Chaieb L, Antal A, Walsh V, Paulus W. Frequency-dependent electrical stimulation of the visual cortex. *Curr Biol*. (2008) 18:1839–43. doi: 10.1016/j.cub.2008.10.027
71. Feurra M, Paulus W, Walsh V, Kanai R. Frequency specific modulation of human somatosensory cortex. *Front Psychol*. (2011) 2:13. doi: 10.3389/fpsyg.2011.00013
72. Santarnecchi E, Polizzotto NR, Godone M, Giovannelli F, Feurra M, Matzen L, et al. Frequency-dependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. *Curr Biol*. (2013) 23:1449–53. doi: 10.1016/j.cub.2013.06.022
73. Makarov S, Horner M, Noetscher G. *Brain and Human Body Modeling*. Cham, CH: Springer (2018). doi: 10.1007/978-3-030-21293-3
74. Voytek B, Kramer MA, Case J, Lepage KQ, Tempesta ZR, Knight RT, et al. Age-related changes in 1/f neural electrophysiological noise. *J Neurosci*. (2015) 35:13257–65. doi: 10.1523/JNEUROSCI.2332-14.2015
75. Peterchev AV, Wagner TA, Miranda PC, Nitsche MA, Paulus W, Lisanby SH, et al. Fundamentals of transcranial electric and magnetic stimulation dose: definition, selection, reporting practices. *Brain Stimul*. (2012) 5:435–53. doi: 10.1016/j.brs.2011.10.001
76. Wagner T, Eden U, Fregni F, Valero-Cabre A, Ramos-Estebanez C, Pronio-Stelluto V, et al. Transcranial magnetic stimulation and brain atrophy: a computer-based human brain model study. *Exp Brain Res*. (2008) 186:539–50. doi: 10.1007/s00221-007-1258-8
77. Wagner T, Fregni F, Fecteau S, Grodzinsky A, Zahn M, Pascual-Leone A. Transcranial direct current stimulation: a computer-based human model study. *Neuroimage*. (2007) 35:1113–24. doi: 10.1016/j.neuroimage.2007.01.027
78. Ruffini G, Wendling F, Sanchez-Todo E, Santarnecchi E. Targeting brain networks with multichannel transcranial current stimulation (tCS). *Curr Opin Biomed Eng*. (2018) 8:70–7. doi: 10.1016/j.cobme.2018.11.001
79. Shulman KI. Clock-drawing: is it the ideal cognitive screening test? *Int J Geriatr Psychiatry*. (2000) 15:548–61. doi: 10.1002/1099-1166(200006)15:6<548::AID-GPS242>3.0.CO;2-U
80. Cohen J, Penney DL, Davis R, Libon DJ, Swenson RA, Ajilore O, et al. Digital clock drawing: differentiating "thinking" versus "doing" in younger and older adults with depression. *J Int Neuropsychol Soc*. (2014) 20:920–8. doi: 10.1017/S1355617714000757
81. Lamar M, Ajilore O, Leow A, Charlton R, Cohen J, GadElkarim J, et al. Cognitive and connectome properties detectable through individual differences in graphomotor organization. *Neuropsychologia*. (2016) 85:301–9. doi: 10.1016/j.neuropsychologia.2016.03.034
82. Papp KV, Rentz DM, Burnham SC, Orlovsky I, Souillard-Mandar W, Penney DL, et al. Digitized clock drawing (DCTCLOCK) performance and its relationship to amyloid and tau pet imaging markers in unimpaired older adults. *Alzheimer's Dementia*. (2018) 14:236–7. doi: 10.1016/j.jalz.2018.06.2374
83. Chapman JE, Cadilhac DA, Gardner B, Ponsford J, Bhalla R, Stolwyk RJ. Comparing face-to-face and videoconference completion of the Montreal Cognitive Assessment (MoCA) in community-based survivors of stroke. *J Telemed Telecare*. (2019). doi: 10.1177/1357633X19890788. [Epub ahead of print].
84. Iiboshi K, Yoshida K, Yamaoka Y, Eguchi Y, Sato D, Kishimoto M, et al. A validation study of the remotely administered montreal cognitive assessment tool in the elderly Japanese population. *Telemed J E Health*. (2020) 26:920–8. doi: 10.1089/tmj.2019.0134

Conflict of Interest: AP-L is a co-founder of Linus Health and TI Solutions AG; serves on the scientific advisory boards for Starlab Neuroscience, Neuroelectrics, Magstim Inc., and MedRhythms; and is listed as an inventor on several issued and pending patents on the real-time integration of non-invasive brain stimulation with electroencephalography and magnetic resonance imaging. MB and GR were employed by Neuroelectrics.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Journal of Psychiatric Research 141 (2021) 248–256

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

tACS as a promising therapeutic option for improving cognitive function in mild cognitive impairment: A direct comparison between tACS and tDCS

Jiheon Kim [a,b], Hansol Kim [b], Hyewon Jeong [b], Daeyoung Roh [a,b], Do Hoon Kim [a,b,*]

[a] Department of Psychiatry, Chuncheon Sacred Heart Hospital, Chuncheon, Republic of Korea
[b] Mind-Neuromodulation Laboratory, College of Medicine, Hallym University, Chuncheon, Republic of Korea

ARTICLE INFO

Keywords:
Transcranial alternating current stimulation
Transcranial direct current stimulation
Neuromodulation
Mild cognitive impairment
Dementia
Electroencephalography

ABSTRACT

Neuromodulation has gained attention as a potential non-pharmacological intervention for mild cognitive impairment (MCI). However, no studies have directly compared the effects of transcranial alternating current stimulation (tACS) with transcranial direct current stimulation (tDCS) on MCI patients. We aimed to identify the more promising and efficient therapeutic option between tACS and tDCS for cognitive enhancement in MCI patients. We compared the effects of gamma-tACS with tDCS on cognitive function and electroencephalography (EEG) in MCI patients. In this sham-controlled, double-blinded, repeated-measures study with the order of the stimulation counterbalanced across patients (n = 20), both gamma-tACS (40 H z) and tDCS were administered at the same intensity (2 mA) in the dorsolateral prefrontal cortex for 30 min. Cognitive tests (Stroop and Trail-Making-Test [TMT]) and EEG were performed before and after single-session stimulation. Gamma-tACS improved the Stroop-color in comparison with tDCS (p = .044) and sham (p = .010) and enhanced the TMT-B in comparison with sham (p = .021). However, tDCS was not significantly different from sham in changes of any cognitive test scores. In EEG analysis, gamma-tACS increased beta activity in comparison with sham and tDCS, whereas tDCS decreased delta and theta activity in comparison with sham. Gamma-tACS also increased beta 2 source activity in the anterior cingulate, compared to sham. The cognitive benefits of tACS in MCI patients appeared superior to those of tDCS. tACS facilitated cognitive function by increasing beta activity, while tDCS delayed the progression of MCI symptoms by decreasing slow-frequency activity. Thus, tACS could be used as a new therapeutic option for MCI.

1. Introduction

Mild cognitive impairment (MCI) is characterized by a cognitive decline to a greater extent than expected for an individual's age and education level such that everyday life is not affected (Winblad et al., 2004). MCI may represent a transitional state between normal aging and dementia (Petersen et al., 1999), and severe cognitive decline in this dementia-related condition could negatively affect independent daily living and social functioning (Lee et al., 2018). Thus, timely and effective interventions are required to improve cognitive function or delay the cognitive decline in patients with MCI (Xu et al., 2019). However, there are still no effective pharmacological treatments that can prevent cognitive symptoms or conversion to dementia (Cooper et al., 2013).

Transcranial electrical stimulation (tES) performed through painless and non-invasive neuro-modulation methods such as transcranial direct current stimulation (tDCS) has increasingly gained attention as a potential non-pharmacological intervention in MCI patients (Canter et al., 2016; Matsumoto and Ugawa, 2017). tDCS modulates spontaneous cortical activity with low-intensity currents (e.g., 1–2 mA), thereby altering plasticity in the stimulated brain regions (Birba et al., 2017). Since an altered dorsolateral prefrontal cortex (DLPFC) is the major neural basis responsible for the cognitive deficit in MCI (Liang et al., 2011; Murugaraja et al., 2017), tDCS over DLPFC could be an effective therapeutic option to modulate functional neural deficits in this pathology (Meinzer et al., 2015; Murugaraja et al., 2017). tDCS also could normalize the pathological electroencephalography (EEG) pattern in

Abbreviations: MCI, mild cognitive impairment; tES, transcranial electrical stimulation; tDCS, transcranial direct current stimulation; tACS, transcranial alternating current stimulation; DLPFC, dorsolateral prefrontal cortex; EEG, electroencephalography; AD, Alzheimer's disease; DSM, diagnostic and statistical manual of mental disorder; ANOVA, analysis of variance; TMT, Trail-Making-Test; sLORETA, standardized low resolution brain electromagnetic tomography.

* Corresponding author. Department of Psychiatry, Chuncheon Sacred Heart Hospital 77, Sakju-ro, Chuncheon-si, Gangwon-do, 24253, Republic of Korea.
E-mail address: dohkim@hallym.ac.kr (D.H. Kim).

https://doi.org/10.1016/j.jpsychires.2021.07.012
Received 31 January 2021; Received in revised form 17 June 2021; Accepted 5 July 2021
Available online 6 July 2021
0022-3956/

MCI (Ferrucci et al., 2018; Marceglia et al., 2016), which is characterized by increased slow-frequency activity and decreased alpha power activity related to cognitive dysfunction (Chiaramonti et al., 1997; Koberda et al., 2013).

A growing body of evidence has shown the effects of tDCS over DLPFC for cognitive enhancement in both healthy individuals and patients with MCI and Alzheimer's disease (AD). However, some recent meta-analyses concluded that tDCS could have mixed, contradictory (Dedoncker et al., 2016; Mancuso et al., 2016; Medina and Cason, 2017; Tremblay et al., 2014), or even minimal to no effects in a healthy population (Horvath et al., 2015). Moreover, for patients with MCI and AD, a meta-analysis of randomized controlled trials on tDCS indicated a lack of clear evidence concerning the cognitive benefits of tDCS (Inagawa et al., 2019). Thus, due to the inadequate and contradictory evidence, it remains unclear whether tDCS could prevent cognitive decline in MCI.

Transcranial alternating current stimulation (tACS) has emerged as a promising alternative treatment option that may be better suited to improve cognitive function. A growing recent body of literature has reported the cognitive enhancement effects of tACS in diverse cognitive domains in mainly healthy populations (Feurra et al., 2016; Nomura et al., 2019; Santarnecchi et al., 2016; van Driel et al., 2015; Yaple et al., 2017). tACS differs from tDCS in that it delivers an electrical current alternating at a specified frequency in a bidirectional manner between the electrodes, while tDCS delivers a unidirectional electrical current (Antal and Paulus, 2013; Helfrich et al., 2014). tACS in the EEG frequency range (e.g., 40 Hz) could lead to entrainment of brain oscillations at the targeted frequency of stimulation (e.g., gamma) (Herrmann et al., 2013). For example, gamma-tACS entrains endogenous oscillations theoretically by altering gamma activity closely linked to cognition (Hermann et al., 2013; Hoy et al., 2015), thereby more directly enhancing cognitive function or modulating the neuropathology of cognitive dysfunction in comparison with tDCS (Hermann et al., 2013; Hoy et al., 2015). Gamma-tACS has been recently reported to enhance the cognitive processes involved in memory, attention, and executive function in healthy populations (Hopfinger et al., 2017; Hoy et al., 2015; Nomura et al., 2019; Santarnecchi et al., 2016, 2019). However, only one study (Hoy et al., 2015) has directly compared the differential effects of tDCS and gamma-tACS on cognitive enhancement (working memory) in healthy subjects. The findings of the study elucidated clear benefits of tACS, compared to tDCS (please refer to (Jones et al., 2019; Röhner et al., 2018) for the results of theta-tACS). Moreover, since abnormal gamma oscillations in the resting state are closely related to cognitive deficits (Babiloni et al., 2013; Hsiao et al., 2014; Moretti et al., 2011; Rossini et al., 2006), a small number of recent studies have shown the therapeutic benefits of gamma-tACS in MCI patients (Naro et al., 2016; Xing et al., 2020).

However, to date, no studies have directly compared the cognitive performance or neurophysiological changes between tDCS and tACS in patients with MCI. This comparison could more objectively demonstrate the potential of tACS as a promising treatment option for cognitive enhancement in comparison with conventional tDCS in patients with pathological aging. In particular, quantitative EEG analysis in the frequency domain can provide information regarding the presence of different oscillations (Marceglia et al., 2016). Moreover, the EEG could reflect fluctuations in resting membrane potential by brain stimulation, and eventually facilitate in vivo determination of brain regions influenced by the stimulation (Azarpaikan et al., 2019; Mangia et al., 2014). Since tDCS and tACS might have substantially different neural mechanisms, as mentioned earlier, an assessment of qEEG modifications in parallel with neurocognitive variables could widen the perspective on the differential neurophysiological effects of tACS and tDCS and their possible relationship with stimulation-induced cognitive benefits.

Thus, the present study aimed to verify the effects of gamma-tACS on cognitive function and electrophysiology in comparison with the effects of tDCS and sham stimulation in MCI patients. We matched the parameters of gamma-tACS and tDCS as closely as possible by using the same current intensity (2 mA, 30 min) within the same target area (right and left DLPFCs). This study used a sham-controlled, double-blinded, repeated-measures design with the order of the stimulation interventions counterbalanced across participants. We made the following hypotheses: (1) gamma-tACS and tDCS would more significantly enhance cognitive test performance than sham stimulation, and the degree of enhancement in gamma-tACS would be superior to that achieved with conventional tDCS; (2) in power spectral analysis for resting-state EEG, gamma-tACS and tDCS would show differential changes in EEG frequency patterns immediately after the stimulation intervention.

2. Material and methods

2.1. Participants

This study was conducted in the psychiatry units at Chuncheon Sacred Heart Hospital, a teaching hospital affiliated with the Hallym University, College of Medicine, Republic of Korea. It was registered with the Clinical Trials Registry Korea (registration: KCT0004105). The present study was approved by the Institutional Review Board of Chuncheon Sacred Heart Hospital, Republic of Korea (Approval No. 2017–82). All participants provided written informed consent. Patients over aged 55 years with MCI were recruited via advertisements at local hospitals with psychiatric units and community mental health centers. The inclusion criteria for the study were as follows: (a) confirmed diagnosis of MCI based on the criteria defined by Petersen (Petersen et al., 1999), namely memory complaint, normal activities of daily living, normal general cognitive function, abnormal memory for age, and absence of dementia; (b) met the DSM-5 criteria (APA, 2000) for mild neurocognitive disorder; and (c) a score of 0.5–1 on the Clinical Dementia Rating (CDR) Scale (Berg, 1984) or a score of 2–4 on the Global Deterioration Scale (GDS) (Reisberg et al., 1988). Participants were excluded if they (a) had other neurological illness or conditions, such as head trauma or Parkinson's disease; (b) had other psychiatric disturbances consistent with specific DSM criteria (APA, 2000), such as substance abuse or dependence; (c) had medical illness that affected brain function; or (d) had cardiovascular disease or an asthmatic condition. Face-to-face diagnostic interviews were conducted by a board-certified clinical psychologist who reviewed the history, symptoms, and psychosocial function of each patient by using all available sources of information. We screened a total 20 participants aged between 71 and 81 years, where all participants met the initial criteria and did not drop out.

2.2. Study procedures

This study employed a sham-controlled, double-blind, repeated-measures design with the order of the stimulation interventions counterbalanced across participants (Fig. 1). Each participant was required to attend three separate sessions. Each session involved either gamma-tACS, tDCS, or sham stimulation. Because an interval of one week between sessions is considered adequate to avoid the carryover effects of stimulation (Andrews et al., 2011), each participant received three interventions on the same weekday for three consecutive weeks. The research assistant counterbalanced the order of stimulation type by using a pseudo-randomization method at the beginning of study.

During each session, resting-state EEG recording and neuropsychological assessments, including evaluations with the Stroop test (Stroop, 1992) and Trail-Making Test (TMT) (Reitan, 1958), were applied before (baseline) and after (post-intervention) the intervention. The Stroop test and TMT are the components of the Seoul Neuropsychological Screening Battery (SNSB) (Ahn et al., 2010) and the clinical assessment battery of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Korean version) (Lee et al., 2002), respectively. Multiple cognitive functions are involved in the Stroop test and TMT (Llinàs-Reglà et al., 2017; Scarpina and Tagini, 2017), and both are representative tests that

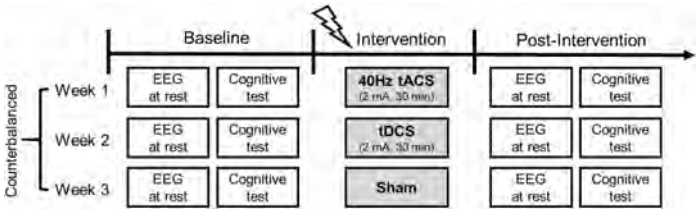

Fig. 1. Timeline of the experimental protocol.

are sensitive to the cognitive changes related to both normal and pathological aging (Bondi et al., 2002; Rasmusson et al., 1998).

2.3. Intervention protocol

The stimulation device used was the MIND-D (YBRAIN, Republic of Korea, http://www.ybrain.com/). Gamma-tACS, tDCS, and the sham devices used the same electrodes (electrode size, 28.26 cm^2; current density, 0.07 mA/cm^2) that were round-sponge patch-soaked in 0.9% sodium chloride (in about 7 mL of saline). All participants sat comfortably on the same chair for the intervention. Both single-session tACS and tDCS interventions were administrated equally at 2 mA for 30 min at the dorsolateral prefrontal cortex (DLPFC), which is located at F3 (left) and F4 (right) based on the extended international 10–20 system. In the tDCS session, bilateral stimulation (anode F3 and cathode F4) were applied based on the previous tDCS studies for cognitive enhancement of MCI or AD patients (Im et al., 2019; Yun et al., 2016). The inhibitory effect of cathode stimulation on cognition insignificant (Jacobson et al., 2012) and bi-hemispheric stimulation may deliver current more deeply and widely due to inter-hemispheric interactions (Kwon and Jang, 2012; Lindenberg et al., 2013). The gamma-tACS protocol consisted of 40 Hz stimulations at the left and right DLPFCs. Tolerability of tACS and tDCS were assessed at the end of each session via self-report following questioning from the research assistant who experienced tES administration.

2.4. EEG acquisition and recording

The resting-state EEG recording were performed with the participant eyes closed over 5 min by using the NetStation 4.4.2 (EGI Software, Eugene, OR, USA), an EGI NetAmps 300 amplifier with a 24-bit analog-to-digital converter, and the HydroCel Geodesic sensor net. The recordings were performed with 59 scalp channels. Additional electrodes were placed below and on the outer canthus of both eyes for vertical and horizontal electrooculography, respectively. Impedance was maintained lower than 50 kΩ. The EEG recordings were bandpass-filtered at 1–100 Hz, at a sampling rate of 1000 Hz. The on-line signals were referenced to CZ where the ground electrode was placed between CZ and PZ.

2.5. EEG processing and analysis

The EEG data were processed and analyzed using Matlab 2017 software (MathWorks, Natick, MA, USA). The data were also checked for gross artifacts by visual inspection and sweeps of the recording by a trained assessor with no prior information of the data. Recordings were re-referenced using common average reference during preprocessing. Power spectral analyses were performed using Fast Fourier Transformation with a 1–50-Hz filter to obtain absolute power values. Frequency bands were defined as follows: delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), low-beta (12–18 Hz), high-beta (18–30 Hz), and gamma (30–50 Hz). Artifacts exceeding ±100 μV were excluded at all channels. Fifteen randomized artifact-free epochs (epoch length: 4096 s) were determined for each participant. The cortical deep source was analyzed using sLORETA (Standardized low resolution brain electromagnetic tomography) software to calculate the EEG inverse solution at cortical source regions based on the 6239 voxels (Pascual–Marqui, 2002).

2.6. Statistical analysis

Statistical analyses were conducted with SPSS version 20.0 (SPSS, Chicago, NJ, USA). The differences in baseline cognitive performance and baseline EEG activity among tACS, tDCS, and sham stimulation were analyzed using repeated-measures ANOVA. To compare differences between changes in cognitive performance and EEG activity among baseline and post-intervention time points for the three interventions, repeated-measure ANOVA was performed with interventions (tACS, tDCS, and sham) as a within-group factor. Changes in the values were calculated by subtracting baseline values from the post-intervention values. Post-hoc pairwise comparisons were performed using Bonferroni corrections. Deep-source images were examined using a statistical non-parametric mapping method provided with the sLORETA software. Paired *t*-statistics were obtained to compare the changes in brain activity (subtract baseline from post-intervention) between the interventions (tACS versus tDCS, tACS versus sham, and tDCS versus sham). The estimated voxel activation was averaged for the calculated time points and tested voxel-by-voxel for the 9239 voxels, followed by adjustments for multiple comparisons (5000 permutations). The level of statistical significance was set at $p < .05$.

3. Results

3.1. Demographics, clinical characteristics, baseline cognitive performance and brain activity

Table 1 summarizes the demographics, clinical characteristics, and performance in the baseline cognitive tests of participants. The repeated measure ANOVA did not reveal any significant differences either in the cognitive tests scores or in the EEG-power values for all electrodes

Table 1
Demographics, clinical data and baseline performance of the study population ($n = 20$).

Variables	Mean	SD
Sex	Male $n = 7$ (35%)	Female $n = 13$ (65%)
Age	76.8	3.2
Years of education	5.6	2.8
CDR	0.5	0
GDS-Dementia	2.8	0.6
GDS-Depression	5.1	3.6
MMSE	20.38	4.6
Baseline Stroop-Word (n)	91.1	24.5
Baseline Stroop-Color (n)	48.7	14.6
Baseline TMT-A (s)	43.4	19.9
Baseline TMT-B (s)	79.3	23.8

SD, Standard deviation; CDR, Clinical Dementia Rating Scale; GDS, Global Deterioration Scale; MMSE, Mini-Mental State Examination; TMT, Trail Making Test; n, number; s, second.

within each frequency band between the aforementioned three interventions (tACS, tDCS, and sham) at baseline.

3.2. Effects of interventions on cognitive function

The repeated-measures ANOVA showed a significant within-subjects effect of intervention (tACS, tDCS, and sham) on changes in the Stroop-color ($F = 6.4$, $p = .004$), and TMT-B ($F = 4.3$, $p = .021$) scores between baseline and post-intervention (Table 2). Post-hoc comparisons (Fig. 2) showed a greater change in the Stroop-color score after tACS in comparison with that after tDCS ($p = .044$) and sham stimulation ($p = .010$). The change in the TMT-B score after tACS was greater than that after the sham stimulation ($p = .021$). However, there was no significant difference between the interventions with respect to changes in the Stroop-word and TMT-A scores.

3.3. Effects of interventions on resting-state EEG

3.3.1. Power spectral analysis

The repeated-measures ANOVA yielded a significant within-subjects effect of intervention (tACS, tDCS, and sham) on EEG-power changes from baseline and post-intervention at the delta, theta, and beta frequency bands (Table 3). In the delta band, post-hoc comparisons showed that tDCS decreased post-intervention activation at the FC4 electrode ($p = .034$) in comparison with the sham stimulation. In the theta band, tDCS decreased post-intervention activation at the F3 ($p = .040$) and FC3 electrodes ($p = .020$) in comparison with the sham stimulation. In the beta band, tACS increased post-intervention activation at the F8 ($p = .031$) and P4 ($p = .041$) electrodes in comparison with tDCS and sham stimulation, respectively (see Fig. 3).

3.3.2. Source localization

In source analysis, tACS showed increased changes in the activity of the anterior cingulate at the beta 2 frequency band between baseline and post-intervention compared to sham stimulation (threshold = 4.6, $p > .05$, voxel-value = 4.8, MNI [x,y,z] = −15, 35, 25) (see Fig. 4).

3.4. Tolerability of interventions

All participants well tolerated both tACS and tDCS. Adverse effects noted during the sessions were mild pricking sensation (tACS: $n = 4$, 20%, tDCS: $n = 2$, 10%). There were no other adverse effects reported.

4. Discussion

To the best of our knowledge, the present study is the first to demonstrate the effectiveness of tACS on both cognitive enhancements and electrophysiology findings through direct comparison with tDCS in MCI patients. Gamma-tACS significantly improved performance of the Stroop-color test, in comparison to tDCS and sham in the cognitive test. In addition, it enhanced TMT-B performance, compared to sham stimulation. However, the changes in cognitive task performance did not substantially vary between the tDCS and sham stimulation. In the power spectral analysis, gamma-tACS increased beta activity in the right frontal and parietal regions in comparison with sham stimulation and tDCS, respectively. tDCS decreased delta activity in the right frontocentral region and theta activity in the left frontal and frontocentral regions in comparison with the sham stimulation. In the source analysis, only tACS increased neural activity at the beta-2 frequency in the anterior cingulate in comparison with the sham stimulation. All participants well tolerated both tACS and tDCS with no adverse effects reported except for mild pricking sensation.

Gamma-tACS provided cognitive benefits to patients with MCI, consistent with our hypothesis, whereas the tDCS did not. The benefits of the former became evident with an increase in difficulty in the cognitive tests, compared to tDCS and sham. These findings elucidate the positive effects of gamma-tACS on higher-level cognitive functions, such as cognitive control, working memory, and decision making. Specifically, unlike Stroop-word reading, which is an over-learned and automatic behavior, the Stroop-color assessment requires attention and inhibitory control to suppress task-irrelevant associations (word reading) (León-Carrion et al., 2008; Lezak et al., 2004). Similarly, TMT-A mainly involves attention, visual perception, and processing speed, but TMT-B further requires working memory, decision-making, and attentional shift to alternate between numbers and letters (Lezak et al., 2004; Sánchez-Cubillo et al., 2009). Although the present study included only two cognitive tests, both of them were quick and concise while also reliably measuring the various cognitive domains, including higher-level cognition for an especially older population (Bondi et al., 2002; Llinàs-Reglà et al., 2017; Rasmusson et al., 1998; Scarpina and Tagini, 2017).

Some recent literature has reported the effects of gamma-tACS on these cognitive processes in mainly normal subjects (Hopfinger et al., 2017; Hoy et al., 2015; Santarnecchi et al., 2013, 2016), indicating its clinical implications for MCI. For instance, one study showed that gamma-tACS improved disengagement from invalid cues in the endogenous attention task, indicating the influence of gamma-tACS on attentional shifting and reorienting (Hopfinger et al., 2017). Although episodic memory deficits are known to be a sensitive cognitive marker during AD progression (Rajah et al., 2017), deficits in working memory and executive function, e.g., poor selective and divided attention or failure to inhibit task-irrelevant stimuli, further characterize MCI or early stages of AD (Kirova et al., 2015). Our findings thus provide evidence for the effects of gamma-tACS on this higher-level cognitive function in MCI patients. tACS could eventually leave room for cognitive restructuring and neuroplasticity on a compensatory basis in MCI patients.

The electrophysiological changes observed after stimulation support the aforementioned cognitive benefits of gamma-tACS in patients with MCI. Contrary to our expectations, our findings showed that gamma-tACS resulted in increased beta power activity, not gamma activity. Thus, gamma-tACS does not appear to lead to entrainment of endogenous oscillation at the 40-Hz frequency in the present study. Rather, plastic alterations within the stimulated area or network are believed to have contributed to these findings (Moliadze et al., 2019; Wach et al., 2013). Consistent with our findings, recent research showed that tACS does not necessarily cause after-effects at the stimulation frequency, but selectively causes them at a different frequency band (Chaieb et al., 2014; Moliadze et al., 2019; Wach et al., 2013). A study by Moliadze and colleagues found that alpha-tACS (10 Hz) not only facilitated phonological response speed, but also increased theta, not alpha, power during phonological decisions (Moliadze et al., 2019). Wach and colleagues

Table 2
Within-subject effect of intervention (tACS, tDCS and Sham) in changes of cognitive tests scores from baseline and post-intervention.

Variable	Changes in performance			F	*P*	η^2	*Post hoc*
	tACS	tDCS	Sham				
Stroop-Word (n)	−1.8 (9.5)	−4.0 (11.7)	−2.8 (12.0)	0.2	.801	.014	–
Stroop-Color (n)	17.6 (11.8)	8.6 (10.5)	5.7 (9.1)	6.4	.004	.251	tACS > Sham tACS > tDCS
TMT-A (s)	−6.9 (6.2)	−6.7 (10.3)	−2.5 (12.3)	1.6	.217	.086	–
TMT-B (s)	−23.8 (17.1)	−23.1 (15.4)	−12.8 (13.1)	4.3	.021	.184	tACS > Sham

TMT, Trail Making Test; n, number; s, second.
Note: Repeated measures ANOVA was performed with interventions as a within-group factor. Post-hoc pairwise comparisons were performed using Bonferroni corrections.

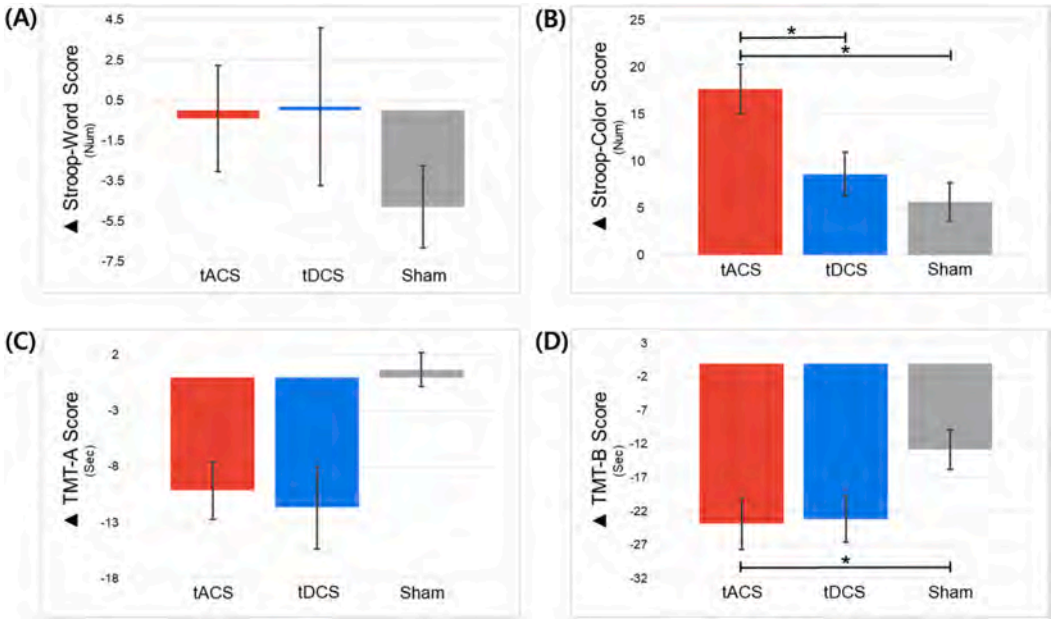

Fig. 2. Bonferroni post-hoc comparison of differences in the changes in the (▲) Stroop and trail-making test (TMT) scores from baseline and post-intervention between interventions, $*p < .05$ and $**p < .01$.

Table 3
Within-subject effect of intervention (tACS, tDCS and Sham) in changes of EEG-power values (μV^2) from baseline and post-intervention.

Band	Channel	Changes in EEG Value			F	*P*	η^2	*Post hoc*
		tACS	tDCS	Sham				
Delta	Fc4	−0.1 (0.6)	−0.2 (0.5)	0.2 (0.5)	4.8	.015	.231	tDCS < Sham
	Oz	0.3 (1.1)	−0.5 (0.8)	0.2 (0.8)	3.4	.047	.174	Non-sig
Theta	F3	0.3 (0.6)	−0.3 (0.9)	−0.2 (0.5)	3.4	.045	.176	tDCS < Sham
	F8	0.8 (1.0)	0.2 (0.5)	0.1 (0.6)	5.0	.013	.238	Non-sig
	Fc3	0.2 (0.5)	−0.2 (0.6)	0.2 (0.4)	3.9	.031	.196	tDCS < Sham
	C2	0.3 (0.5)	−0.2 (0.7)	0.0 (0.5)	4.8	.015	.231	Non-sig
	C4	0.3 (0.4)	−0.1 (0.5)	0.1 (0.5)	3.3	.048	.173	Non-sig
	C5	0.1 (0.5)	−0.2 (0.5)	0.2 (0.5)	3.6	.039	.184	Non-sig
	C6	0.5 (0.6)	0 (0.5)	0.1 (0.5)	3.7	.035	.189	Non-sig
	Cp2	0.4 (0.5)	−0.1 (0.5)	0.1 (0.5)	4.3	.023	.211	Non-sig
	P4	0.4 (0.5)	−0.2 (0.6)	0.3 (0.7)	4.1	.026	.203	Non-sig
Beta	F8	0.3 (0.3)	0.0 (0.3)	−0.1 (0.4)	4.9	.014	.233	tACS > Sham
	P4	−0.1 (0.6)	−0.2 (0.5)	0.2 (0.5)	4.5	.019	.221	tACS > tDCS

Note: Repeated measures ANOVA was performed with interventions as a within-group factor. Post-hoc pairwise comparisons were performed using Bonferroni corrections.

also reported that beta-tACS (20 Hz) caused a reduction in cortico-muscular coherence amplitude in the low gamma band, but not in the beta band (Wach et al., 2013). In other words, tACS may induce plastic alterations to yield offline effects of stimulation rather than entrainment of cortical oscillations (Wach et al., 2013).

The discriminatory electrophysiological effects of each tES in the present study were that tACS increased beta activity in mainly the frontal region with sub-cortical brain activity at the anterior cingulate, which is closely linked to cognitive function, while tDCS reduced slow-frequency activity representing the pathological EEG pattern of MCI. The beta activity facilitated by tACS in the present study is known to play a critical role in cognitive processes, particularly in relation to the attentional system (Gola et al., 2013). In particular, beta oscillations in the frontal cortex and anterior cingulate with the cortical and sub-cortical areas have been identified as a key position for top-down processing of cognition, contributing to the regulation of cognitive control (Devinsky et al., 1995; Løvstad et al., 2012; Siegel et al., 2012; Stoll et al., 2016). The computational-level mechanisms of cognitive control involve variable mediation of cognitive-level functions such as working memory and attention (Stoll et al., 2016). Frontal beta oscillations also play a role in interacting with other cortical regions, for example, by enhancing frontal and parietal beta coherence during decision-making (Pesaran et al., 2008). Additionally, the most well-studied paradigm in the functional neuroimaging of the anterior cingulate was found to be Stroop or Stroop-like tasks (Mansouri et al., 2009). Based on this evidence, our study suggests a potential linkage

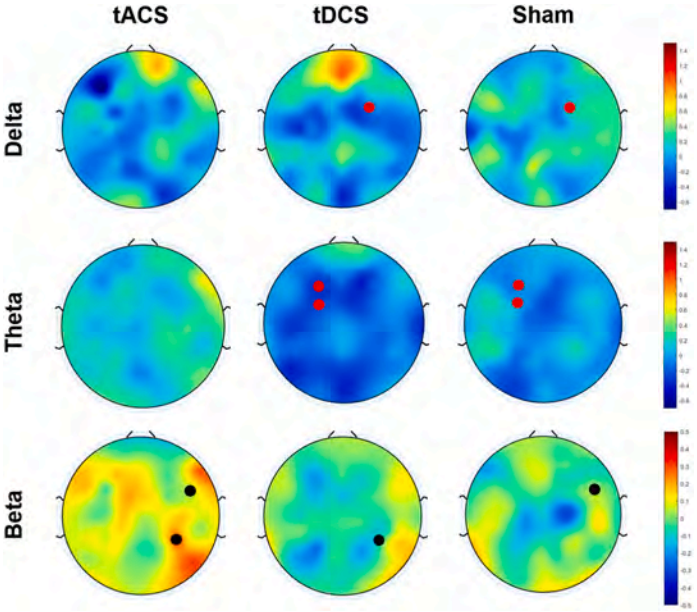

Fig. 3. Bonferroni post-hoc comparison of differences in the changes in EEG-power activity from baseline and post-intervention between interventions. Significant differences between interventions were marked. The red dots indicate channels with significant differences in EEG activity between tDCS and sham simulation. The black dots indicate channels with significant differences in EEG activity between tACS and other stimulations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

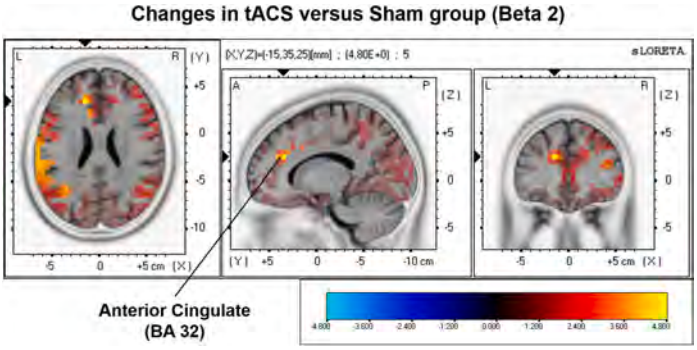

Fig. 4. sLORETA imaging results of resting-state EEG supra-threshold voxels in the anterior cingulate at the beta 2 frequency band illustrating increased neuronal activity in tACS at post-intervention compared to sham stimulation. BA, Brodmann area.

between cognitive facilitation and the EEG fluctuations modulated by tACS.

Despite the absence of cognitive effectiveness, tDCS over DLPFC primarily reduced slow-frequency activity mainly in the frontal brain regions. Previous studies have reported about a slow oscillation-modulating effect of tDCS in patients with dementia (Ferrucci et al., 2018; Marceglia et al., 2016). According to Ferrucci and colleagues, bilateral anodal tDCS over the frontotemporal cortex led to a decrease in qEEG patterns in the delta and theta frequency in frontotemporal dementia. This, in turn, was correlated with the neuropsychiatric signs showing improvement in dementia (Ferrucci et al., 2018). Aging is concomitant with an enhancement of resting-state activity in the slow-frequency range (Hoyer et al., 2004). In addition, abnormal activity of this oscillation is a potent electrophysiological indicator of pathological aging (Chiaramonti et al., 1997; Koberda et al., 2013). Considering the association between cognitive processing and the integrity and efficiency of the frontal brain (Haworth et al., 2016; Kochunov et al., 2010), altered frontal functioning, including DLPFC, in the slow oscillations is a possible neural basis for cognitive decline in MCI (Liang et al., 2011; Murugaraja et al., 2017; Yang et al., 2009). Despite the absence of any tDCS-mediated cognition facilitating effect, our findings shed light on its therapeutic potential for the management of MCI. However, its electrophysiological modulatory mechanisms could possibly differ from those of tACS. Future studies should examine the impact of long-term tDCS on the transition of MCI to dementia.

This study presents the differential stimulation-induced cognitive benefits and their potential relationship with electrophysiological patterns generated by gamma-tACS and tDCS. Gamma-tACS could facilitate

cognitive function more directly by increasing beta frequency activity, whereas tDCS prevented or delayed the progression of MCI symptoms by decreasing the slow-frequency activity. This study also suggests that in this modulatory process, the cognitive benefits of tACS is more likely to be superior that of tDCS in MCI patients. MCI represents a critical stage room for cognitive restriction and neuroplasticity based on compensatory mechanisms (Kirova et al., 2015). Therefore, the positive effects of gamma-tACS, especially on higher-level cognitive function, have important and promising therapeutic implications for MCI patients.

There are limitations to the present findings that warrant discussion. First, the major limitation of the study is that the tES session was administered only once. Multi-session tDCS particularly was reported to improve cognitive functions in some major psychiatric and neurological illnesses, e.g., schizophrenia (Narita et al., 2020) and stroke (Yan et al., 2020). Lefaucheur and colleagues suggest the application of a single session might be less effective than a longer exposure to tDCS (Lefaucheur et al., 2017). Thus, further multi-session study is warranted to verify the possible benefits of tDCS and tACS on other cognitive domains in MCI patients. Second, although both TMT and Stroop test are reliable for measuring various cognitive domains in the elderly (Bondi et al., 2002; Llinàs-Reglà et al., 2017; Rasmusson et al., 1998; Scarpina and Tagini, 2017), additional measures of cognitive function could help identify the differential cognitive effects between tACS and tDCS more specifically. Third, it is possible that practice effects occurred since participants performed cognitive tasks twice at baseline and post-intervention. However, statistical comparison with sham stimulation validated the significant effectiveness of tES. Fourth, the generalizability of our findings may be limited due to the small sample size. Additional research with between-group comparisons and larger sample sizes are required to address this limitation.

Funding

This research was supported by 1) the Hallym University Research Fund, 2) the National Research Foundation of Korea (NRF) grant funded by the Ministry of Science, ICT, and Future Planning (MSIP), Government of Korea (no. 2017R1A2B4008920), 3) the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2021R1I1A3058026), and 4) the 'R&D Program for Forest Science Technology (Project No. 2021402B10-2123-0101)' provided by Korea Forest Service (Korea Forestry Promotion Institute).

Declaration of competing interests

The authors declare that they have no competing interests.

CRediT authorship contribution statement

Jiheon Kim: Conceptualization, Formal analysis, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Hansol Kim:** Investigation, Data curation, Software, Formal analysis. **Hyewon Jeong:** Investigation, Diagnostic assessment. **Daeyoung Roh:** Resources, Methodology, Validation, Supervision. **Do Hoon Kim:** Conceptualization, Formal analysis, Methodology, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Acknowledgement

None.

References

Ahn, H.J., Chin, J., Park, A., Lee, B.H., Suh, M.K., Seo, S.W., Na, D.L., 2010. Seoul Neuropsychological Screening Battery-dementia version (SNSB-D): a useful tool for assessing and monitoring cognitive impairments in dementia patients. J. Kor. Med. Sci. 25 (7), 1071–1076. https://doi.org/10.3346/jkms.2010.25.7.1071.

Andrews, S.C., Hoy, K.E., Enticott, P.G., Daskalakis, Z.J., Fitzgerald, P.B., 2011. Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. Brain Stimul 4 (2), 84–89. https://doi.org/10.1016/j.brs.2010.06.004.

Antal, A., Paulus, W., 2013. Transcranial alternating current stimulation (tACS). Front. Hum. Neurosci. 7, 317. https://doi.org/10.3389/fnhum.2013.00317.

American Psychiatric Association, 2000. Diagnostic and Statistical Manual of Mental Disorders: DSM-4. Text Rev. American Psychiatric Association, Washington, DC.

Azarpaikan, A., Taherii Torbati, H., Sohrabi, M., Boostani, R., Ghoshuni, M., 2019. Power spectral parameter variations after transcranial direct current stimulation in a bimanual coordination task. Adapt. Behav. 1059712319879971 https://doi.org/10.1177/1059712319879971.

Babiloni, C., Carducci, F., Lizio, R., Vecchio, F., Baglieri, A., Bernardini, S., Frisoni, G.B., 2013. Resting state cortical electroencephalographic rhythms are related to gray matter volume in subjects with mild cognitive impairment and Alzheimer's disease. Hum. Brain Mapp. 34 (6), 1427–1446. https://doi.org/10.1002/hbm.22005.

Berg, L., 1984. Clinical dementia rating. Br. J. Psychiat. 145 (3) https://doi.org/10.1192/S0007125000118082, 339-339.

Birba, A., Ibáñez, A., Sedeño, L., Ferrari, J., García, A.M., Zimerman, M., 2017. Non-invasive brain stimulation: a new strategy in mild cognitive impairment? Front. Aging Neurosci. 9, 16. https://doi.org/10.3389/fnagi.2017.00016.

Bondi, M.W., Serody, A.B., Chan, A.S., Eberson-Shumate, S.C., Delis, D.C., Hansen, L.A., Salmon, D.P., 2002. Cognitive and neuropathologic correlates of Stroop Color-Word Test performance in Alzheimer's disease. Neuropsychol 16 (3), 335. https://doi.org/10.1037/0894-4105.16.3.335.

Canter, R.G., Penney, J., Tsai, L.-H., 2016. The road to restoring neural circuits for the treatment of Alzheimer's disease. Nature 539 (7628), 187–196. https://doi.org/10.1038/nature20412.

Chaieb, L., Antal, A., Pisoni, A., Saiote, C., Opitz, A., Ambrus, G.G., Paulus, W., 2014. Safety of 5 kHz tACS. Brain Stimul 7 (1), 92–96. https://doi.org/10.1016/j.brs.2013.08.004.

Chiaramonti, R., Muscas, G., Paganini, M., Müller, T.J., Fallgatter, A., Versari, V., Strik, W.K., 1997. Correlations of topographical EEG features with clinical severity in mild and moderate dementia of Alzheimer type. Neuropsychobiology 36 (3), 153–158. https://doi.org/10.1159/000119375.

Cooper, C., Li, R., Lyketsos, C., Livingston, G., 2013. A systematic review of treatments for mild cognitive impairment. Br. J. Psychiat. 203 (3), 255. https://doi.org/10.1192/bjp.bp.113.127811.

Dedoncker, J., Brunoni, A.R., Baeken, C., Vanderhasselt, M.A., 2016. The effect of the interval-between-sessions on prefrontal transcranial direct current stimulation (tDCS) on cognitive outcomes: a systematic review and meta-analysis. J. Neural. Transm. 123 (10), 1159–1172. https://doi.org/10.1007/s00702-016-1558-x.

Devinsky, O., Morrell, M.J., Vogt, B.A., 1995. Contributions of anterior cingulate cortex to behaviour. Brain 118 (1), 279–306. https://doi.org/10.1093/brain/118.1.279.

Ferrucci, R., Mrakic-Sposta, S., Gardini, S., Ruggiero, F., Vergari, M., Mameli, F., Marceglia, S., 2018. Behavioral and neurophysiological effects of transcranial direct current stimulation (tDCS) in fronto-temporal dementia. Front. Behav. Neurosci. 12, 235. https://doi.org/10.3389/fnbeh.2018.00235.

Feurra, M., Galli, G., Pavone, E.F., Rossi, A., Rossi, S., 2016. Frequency-specific insight into short-term memory capacity. J. Neurophysiol. 116 (1), 153–158. https://doi.org/10.1152/jn.01080.2015.

Gola, M., Magnuski, M., Szumska, I., Wróbel, A., 2013. EEG beta band activity is related to attention and attentional deficits in the visual performance of elderly subjects. Int. J. Psychophysiol. 89 (3), 334–341. https://doi.org/10.1016/j.ijpsycho.2013.05.007.

Haworth, J., Phillips, M., Newson, M., Rogers, P.J., Torrens-Burton, A., Tales, A., 2016. Measuring information processing speed in mild cognitive impairment: clinical versus research dichotomy. J. Alzheimers Dis. 51 (1), 263–275. https://doi.org/10.3233/JAD-150791.

Helfrich, R.F., Schneider, T.R., Rach, S., Trautmann-Lengsfeld, S.A., Engel, A.K., Herrmann, C.S., 2014. Entrainment of brain oscillations by transcranial alternating current stimulation. Curr. Biol. 24 (3), 333–339. https://doi.org/10.1016/j.cub.2013.12.041.

Herrmann, C.S., Rach, S., Neuling, T., Strüber, D., 2013. Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. Front. Hum. Neurosci. 7, 279. https://doi.org/10.3389/fnhum.2013.00279.

Hopfinger, J.B., Parsons, J., Fröhlich, F., 2017. Differential effects of 10-Hz and 40-Hz transcranial alternating current stimulation (tACS) on endogenous versus exogenous attention. Cognit. Neurosci. 8 (2), 102–111. https://doi.org/10.1080/17588928.2016.1194261.

Horvath, J.C., Forte, J.D., Carter, O., 2015. Quantitative review finds no evidence of cognitive effects in healthy populations from single-session transcranial direct current stimulation (tDCS). Brain Stimul 8 (3), 535–550. https://doi.org/10.1016/j.brs.2015.01.400.

Hoy, K.E., Bailey, N., Arnold, S., Windsor, K., John, J., Daskalakis, Z.J., Fitzgerald, P.B., 2015. The effect of γ-tACS on working memory performance in healthy controls. Brain Cognit. 101, 51–56. https://doi.org/10.1016/j.bandc.2015.11.002.

Hoyer, W.J., Stawski, R.S., Wasylyshyn, C., Verhaeghen, P., 2004. Adult age and digit symbol substitution performance: a meta-analysis. Psychol. Aging 19 (1), 211. https://doi.org/10.1037/0882-7974.19.1.211.

Hsiao, F.J., Chen, W.T., Wang, Y.J., Yan, S.H., Lin, Y.Y., 2014. Altered source-based EEG coherence of resting-state sensorimotor network in early-stage Alzheimer's disease compared to mild cognitive impairment. Neurosci. Lett. 558, 47–52. https://doi.org/10.1016/j.neulet.2013.10.056.

Non-invasive electrical stimulation for the treatment of Alzheimer's disease CLINICAL TRIALS

Im, J.J., Jeong, H., Bikson, M., Woods, A.J., Unal, G., Oh, J.K., Chung, Y.A., 2019. Effects of 6-month at-home transcranial direct current stimulation on cognition and cerebral glucose metabolism in Alzheimer's disease. Brain Stimul 12 (5), 1222–1228. https://doi.org/10.1016/j.brs.2019.06.003.

Inagawa, T., Narita, Z., Sugawara, N., Maruo, K., Stickley, A., Yokoi, Y., Sumiyoshi, T., 2019. A meta-analysis of the effect of multisession transcranial direct current stimulation on cognition in dementia and mild cognitive impairment. Clin. EEG Neurosci. 50 (4), 273–282. https://doi.org/10.1177/1550059418800889.

Jacobson, L., Koslowsky, M., Lavidor, M., 2012. tDCS polarity effects in motor and cognitive domains: a meta-analytical review. Exp. Brain Res. 216 (1), 1–10. https://doi.org/10.1007/s00221-011-2891-9.

Jones, K.T., Arciniega, H., Berryhill, M.E., 2019. Replacing tDCS with theta tACS provides selective, but not general WM benefits. Brain Res. 1720, 146324. https://doi.org/10.1016/j.brainres.2019.146324.

Kirova, A.M., Bays, R.B., Lagalwar, S., 2015. Working memory and executive function decline across normal aging, mild cognitive impairment, and Alzheimer's disease. BioMed Res. Int. 15 https://doi.org/10.1155/2015/748212, 2015.

Koberda, J.L., Moses, A., Koberda, P., Koberda, L., 2013. Clinical advantages of quantitative electroencephalogram (QEEG)–electrical neuroimaging application in general neurology practice. Clin. EEG Neurosci. 44 (4), 273–285. https://doi.org/10.1177/1550059412475291.

Kochunov, P., Coyle, T., Lancaster, J., Robin, D.A., Hardies, J., Kochunov, V., Fox, P.T., 2010. Processing speed is correlated with cerebral health markers in the frontal lobes as quantified by neuroimaging. Neuroimage 49 (2), 1190–1199. https://doi.org/10.1016/j.neuroimage.2009.09.052.

Kwon, Y.H., Jang, S.H., 2012. Onsite-effects of dual-hemisphere versus conventional single-hemisphere transcranial direct current stimulation: a functional MRI study. Neural Regen. Res. 7 (24), 1889. https://doi.org/10.3969/j.issn.1673-5374.2012.24.007.

Lee, C.Y., Cheng, S.J., Lin, H.C., Liao, Y.L., Chen, P.H., 2018. Quality of life in patients with dementia with lewy bodies. Behav. Neurol. 18 https://doi.org/10.1155/2018/8320901, 2018.

Lee, J.H., Lee, K.U., Lee, D.Y., Kim, K.W., Jhoo, J.H., Kim, J.H., Woo, J.I., 2002. Development of the Korean version of the Consortium to establish a Registry for alzheimer's disease assessment packet (CERAD-K) clinical and neuropsychological assessment batteries. J. Gerontol. 57 (1), P47–P53. https://doi.org/10.1093/geronb/57.1.P47.

Lefaucheur, J.P., Antal, A., Ayache, S.S., Benninger, D.H., Brunelin, J., Cogiamanian, F., Paulus, W., 2017. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). Clin. Neurophysiol. 128 (1), 56–92. https://doi.org/10.1016/j.clinph.2016.10.087.

León-Carrion, J., Damas-López, J., Martín-Rodríguez, J.F., Domínguez-Roldán, J.M., Murillo-Cabezas, F., Martin, J.M.B., Domínguez-Morales, M.R., 2008. The hemodynamics of cognitive control: the level of concentration of oxygenated hemoglobin in the superior prefrontal cortex varies as a function of performance in a modified Stroop task. Behav. Brain Res. 193 (2), 248–256. https://doi.org/10.1016/j.bbr.2008.06.013.

Lezak, M.D., Howieson, D.B., Loring, D.W., Fischer, J.S., 2004. Neuropsychological Assessment. Oxford University Press, USA.

Liang, P., Wang, Z., Yang, Y., Jia, X., Li, K., 2011. Functional disconnection and compensation in mild cognitive impairment: evidence from DLPFC connectivity using resting-state fMRI. PloS One 6 (7), e22153. https://doi.org/10.1371/journal.pone.0022153.

Lindenberg, R., Nachtigall, L., Meinzer, M., Sieg, M.M., Flöel, A., 2013. Differential effects of dual and unihemispheric motor cortex stimulation in older adults. J. Neurosci. 33 (21), 9176–9183. https://doi.org/10.1523/JNEUROSCI.0055-13.2013.

Llinàs-Reglà, J., Vilalta-Franch, J., López-Pousa, S., Calvó-Perxas, L., Torrents Rodas, D., Garre-Olmo, J., 2017. The trail making test: association with other neuropsychological measures and normative values for adults aged 55 years and older from a Spanish-Speaking Population-based sample. Assessment 24 (2), 183–196. https://doi.org/10.1177/1073191115602552.

Løvstad, M., Funderud, I., Meling, T., Krämer, U., Voytek, B., Due-Tønnessen, P., Solbakk, A.K., 2012. Anterior cingulate cortex and cognitive control: neuropsychological and electrophysiological findings in two patients with lesions to dorsomedial prefrontal cortex. Brain Cognit. 80 (2), 237–249. https://doi.org/10.1016/j.bandc.2012.07.008.

Mancuso, L.E., Ilieva, I.P., Hamilton, R.H., Farah, M.J., 2016. Does transcranial direct current stimulation improve healthy working memory?: a meta-analytic review. J. Cognit. Neurosci. 28 (8), 1063–1089. https://doi.org/10.1162/jocn_a_00956.

Mangia, A.L., Pirini, M., Cappello, A., 2014. Transcranial direct current stimulation and power spectral parameters: a tDCS/EEG co-registration study. Front. Hum. Neurosci. 8, 601. https://doi.org/10.3389/fnhum.2014.00601.

Mansouri, F.A., Tanaka, K., Buckley, M.J., 2009. Conflict-induced behavioural adjustment: a clue to the executive functions of the prefrontal cortex. Nat. Rev. Neurosci. 10 (2), 141–152. https://doi.org/10.1038/nrn2538.

Marceglia, S., Mrakic-Sposta, S., Rosa, M., Ferrucci, R., Mameli, F., Vergari, M., Priori, A., 2016. Transcranial direct current stimulation modulates cortical neuronal activity in Alzheimer's disease. Front. Neurosci. 10, 134. https://doi.org/10.3389/fnins.2016.00134.

Matsumoto, H., Ugawa, Y., 2017. Adverse events of tDCS and tACS: a review. Clin. Neurophysiol. Pract. 2, 19–25. https://doi.org/10.1016/j.cnp.2016.12.003.

Medina, J., Cason, S., 2017. No evidential value in samples of transcranial direct current stimulation (tDCS) studies of cognition and working memory in healthy populations. Cortex 94, 131–141. https://doi.org/10.1016/j.cortex.2017.06.021.

Meinzer, M., Lindenberg, R., Phan, M.T., Ulm, L., Volk, C., Flöel, A., 2015. Transcranial direct current stimulation in mild cognitive impairment: behavioral effects and neural mechanisms. Alzheimers Dement 11 (9), 1032–1040. https://doi.org/10.1016/j.jalz.2014.07.159.

Moliadze, V., Sierau, L., Lyzhko, E., Stenner, T., Werchowski, M., Siniatchkin, M., Hartwigsen, G., 2019. After-effects of 10 Hz tACS over the prefrontal cortex on phonological word decisions. Brain Stimul 12 (6), 1464–1474. https://doi.org/10.1016/j.brs.2019.06.021.

Moretti, D.V., Frisoni, G.B., Binetti, G., Zanetti, O., 2011. Anatomical substrate and scalp EEG markers are correlated in subjects with cognitive impairment and Alzheimer's disease. Front. Psychiatr. 1, 152. https://doi.org/10.3389/fpsyt.2010.00152.

Murugaraja, V., Shivakumar, V., Sivakumar, P.T., Sinha, P., Venkatasubramanian, G., 2017. Clinical utility and tolerability of transcranial direct current stimulation in mild cognitive impairment. Asian J. Psychiatr. 30, 135–140. https://doi.org/10.1016/j.ajp.2017.09.001.

Narita, Z., Stickley, A., DeVylder, J., Yokoi, Y., Inagawa, T., Yamada, Y., Sumiyoshi, T., 2020. Effect of multi-session prefrontal transcranial direct current stimulation on cognition in schizophrenia: a systematic review and meta-analysis. Schizophr. Res. 216, 367–373. https://doi.org/10.1016/j.schres.2019.11.011.

Naro, A., Corallo, F., De Salvo, S., Marra, A., Di Lorenzo, G., Muscara, N., Calabro, R.S., 2016. Promising role of neuromodulation in predicting the progression of mild cognitive impairment to dementia. J. Alzheimers Dis. 53 (4), 1375–1388. https://doi.org/10.3233/JAD-160305.

Nomura, T., Asao, A., Kumasaka, A., 2019. Transcranial alternating current stimulation over the prefrontal cortex enhances episodic memory recognition. Exp. Brain Res. 237 (7), 1709–1715. https://doi.org/10.1007/s00221-019-05543-w.

Pascual-Marqui, R.D., 2002. Standardized low-resolution brain electromagnetic tomography (sLORETA): technical details. Methods Find. Exp. Clin. Pharmacol. 24 (Suppl. D), 5–12.

Pesaran, B., Nelson, M.J., Andersen, R.A., 2008. Free choice activates a decision circuit between frontal and parietal cortex. Nature 453 (7193), 406–409. https://doi.org/10.1038/nature06849.

Petersen, R.C., Smith, G.E., Waring, S.C., Ivnik, R.J., Tangalos, E.G., Kokmen, E., 1999. Mild cognitive impairment: clinical characterization and outcome. Arch. Neurol. 56 (3), 303–308. https://doi.org/10.1001/archneur.56.3.303.

Rajah, M.N., Wallace, L.M., Ankudowich, E., Yu, E., Swierkot, A., Patel, R., Pasvanis, S., 2017. Family history and APOE4 risk for Alzheimer's disease impact the neural correlates of episodic memory by early midlife. Neuroimage: Clinical 14, 760–774. https://doi.org/10.1016/j.nicl.2017.03.016.

Rasmusson, X.D., Zonderman, A.B., Kawas, C., Resnick, S.M., 1998. Effects of age and dementia on the trail making test. Clin. Neuropsychol. 12 (2), 169–178. https://doi.org/10.1076/clin.12.2.169.2005.

Reisberg, B., Ferris, S.H., De Leon, M., Crook, T., 1988. Global deterioration scale (GDS). Psychopharmacol. Bull. 24 (4), 661–663.

Reitan, R.M., 1958. Validity of the Trail Making Test as an indicator of organic brain damage. Percept. Mot. Skills 8 (3), 271–276.

Röhner, F., Breitling, C., Rufener, K.S., Heinze, H.-J., Hinrichs, H., Krauel, K., Sweeney-Reed, C.M., 2018. Modulation of working memory using transcranial electrical stimulation: a direct comparison between TACS and TDCS. Front. Neurosci. 12, 761. https://doi.org/10.3389/fnins.2018.00761.

Rossini, P., Del Percio, C., Pasqualetti, P., Cassetta, E., Binetti, G., Dal Forno, G., Babiloni, C., 2006. Conversion from mild cognitive impairment to Alzheimer's disease is predicted by sources and coherence of brain electroencephalography rhythms. Neuroscience 143 (3), 793–803. https://doi.org/10.1016/j.neuroscience.2006.08.049.

Sánchez-Cubillo, I., Periáñez, J., Adrover-Roig, D., Rodríguez-Sánchez, J., Rios-Lago, M., Tirapu, J., Barcelo, F., 2009. Construct validity of the Trail Making Test: role of task-switching, working memory, inhibition/interference control, and visuomotor abilities. J. Int. Neuropsychol. Soc. 15 (3), 438. https://doi.org/10.1017/S1355617709090626.

Santarnecchi, E., Muller, T., Rossi, S., Sarkar, A., Polizzotto, N., Rossi, A., Kadosh, R.C., 2016. Individual differences and specificity of prefrontal gamma frequency-tACS on fluid intelligence capabilities. Cortex 75, 33–43. https://doi.org/10.1016/j.cortex.2015.11.003.

Santarnecchi, E., Polizzotto, N.R., Godone, M., Giovannelli, F., Feurra, M., Matzen, L., Rossi, S., 2013. Frequency-dependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. Curr. Biol. 23 (15), 1449–1453. https://doi.org/10.1016/j.cub.2013.06.022.

Santarnecchi, E., Sprugnoli, G., Bricolo, E., Costantini, G., Liew, S.-L., Musaeus, C.S., Rossi, S., 2019. Gamma tACS over the temporal lobe increases the occurrence of Eureka! moments. Sci. Rep. 9 (1), 1–12. https://doi.org/10.1038/s41598-019-42192-z.

Scarpina, F., Tagini, S., 2017. The stroop color and word test. Front. Psychol. 8, 557. https://doi.org/10.3389/fpsyg.2017.00557.

Siegel, M., Donner, T.H., Engel, A.K., 2012. Spectral fingerprints of large-scale neuronal interactions. Nat. Rev. Neurosci. 13 (2), 121–134. https://doi.org/10.1038/nrn3137.

Stoll, F.M., Wilson, C.R., Faraut, M.C., Vezoli, J., Knoblauch, K., Procyk, E., 2016. The effects of cognitive control and time on frontal beta oscillations. Cerebr. Cortex 26 (4), 1715–1732. https://doi.org/10.1093/cercor/bhv006.

Stroop, J.R., 1992. Studies of interference in serial verbal reactions. J. Exp. Psychol. Gen. 121 (1), 15. https://doi.org/10.1037/0096-3445.121.1.15.

Tremblay, S., Lepage, J.-F., Latulipe-Loiselle, A., Fregni, F., Pascual-Leone, A., Théoret, H., 2014. The uncertain outcome of prefrontal tDCS. Brain Stimul 7 (6), 773–783. https://doi.org/10.1016/j.brs.2014.10.003.

van Driel, J., Sligte, I.G., Linders, J., Elport, D., Cohen, M.X., 2015. Frequency band-specific electrical brain stimulation modulates cognitive control processes. PloS One 10 (9), e0138984. https://doi.org/10.1371/journal.pone.0138984.

Wach, C., Krause, V., Moliadze, V., Paulus, W., Schnitzler, A., Pollok, B., 2013. The effect of 10 Hz transcranial alternating current stimulation (tACS) on corticomuscular coherence. Front. Hum. Neurosci. 7, 511. https://doi.org/10.3389/fnhum.2013.00511.

Winblad, B., Palmer, K., Kivipelto, M.J., Jelic, V.V., Fratiglioni, L., Wahlund, L.O., Petersen, R.C., 2004. Mild cognitive impairment—beyond controversies, towards a consensus: report of the international working group on mild cognitive impairment. J. Intern. Med. 256 (3), 240–246. https://doi.org/10.1111/j.1365-2796.2004.01380.x.

Xing, Y., Wei, P., Wang, C., Shan, Y., Yu, Y., Qiao, Y., Tang, Y., 2020. Transcranial alternating current stimulation for patients with mild Alzheimer's disease (TRANSFORM-AD study): protocol for a randomized controlled clinical trial. Alzheimers Dement 6 (1), e12005. https://doi.org/10.1002/trc2.12005.

Xu, Y., Qiu, Z., Zhu, J., Liu, J., Wu, J., Tao, J., Chen, L., 2019. The modulation effect of non-invasive brain stimulation on cognitive function in patients with mild cognitive impairment: a systematic review and meta-analysis of randomized controlled trials. BMC Neurosci. 20 (1), 2. https://doi.org/10.1186/s12868-018-0484-2.

Yan, R.B., Zhang, X.L., Li, Y.H., Hou, J.M., Chen, H., Liu, H.L., 2020. Effect of transcranial direct-current stimulation on cognitive function in stroke patients: a systematic review and meta-analysis. PloS One 15 (6), e0233903. https://doi.org/10.1371/journal.pone.0233903.

Yang, Y., Liang, P., Lu, S., Li, K., Zhong, N., 2009. The role of the DLPFC in inductive reasoning of MCI patients and normal agings: an fMRI study. Sci. China Ser. C Life Sci. 52 (8), 789–795. https://doi.org/10.1007/s11427-009-0089-1.

Yaple, Z., Martinez-Saito, M., Awasthi, B., Feurra, M., Shestakova, A., Klucharev, V., 2017. Transcranial alternating current stimulation modulates risky decision making in a frequency-controlled experiment. Eneuro 4 (6). https://doi.org/10.1523/ENEURO.0136-17.2017.

Yun, K., Song, I.U., Chung, Y.A., 2016. Changes in cerebral glucose metabolism after 3 weeks of noninvasive electrical stimulation of mild cognitive impairment patients. Alzheimer's Res. Ther. 8 (1), 1–9. https://doi.org/10.1186/s13195-016-0218-6.

Effects of 40 Hz transcranial alternating current stimulation (tACS) on cognitive functions of patients with Alzheimer's disease: a randomised, double-blind, sham-controlled clinical trial

INTRODUCTION

Gamma frequency stimulation is found to alleviate memory deficits on animal models of Alzheimer's disease (AD), potentially by activating neuroimmune signalling and removing A-beta plaques in the brain.[1 2] A feasible, translational hypothesis is that gamma band brain stimulation (eg, transcranial alternating current stimulation (tACS)) would yield clinical benefits on cognition in patients with AD. Here we performed a 6-week gamma tACS (2 mA, 40 Hz over bilateral temporal lobes) on a total of 50 subjects to elucidate clinical efficiency and safety of gamma stimulation for patients with AD.

METHODS

The study included a 6-week tACS intervention phase (5 days on and 2 days off for weekends) and another 12-week efficacy/safety assessment phase without tACS intervention (figure 1A). Bilateral tACS over temporal lobes (located by 10–20 electroencephalogram system) was delivered through saline-soaked sponges (sized 4×4 cm^2) using a stimulator (Transcranial, London, UK). The 40 Hz sinusoidal current was applied at 2 mA with a duration of 20 min. Mini-Mental State Examination (MMSE) and Alzheimer's Disease Cognitive Component Assessment (ADAS-Cog) were used for cognition evaluation before (0 week), at the end of (6 weeks) and 12 weeks (end +12 weeks) after the end of the stimulation. Serum A-beta levels were measured with ELISA.

SPSS software was used to generate a list of random numbers, followed by dividing all patients with AD who fit into the group into a real stimulation group and a sham stimulation group at a ratio of 1:1. All tACS treatment devices had the same appearance and colour. Throughout the experiment, the patients did not know what kind of treatment they received, and the operators did not know the patients' enrolment and allocation either. All the scales were independently evaluated by the full-time staff that had been blinded to the grouping of the subjects.

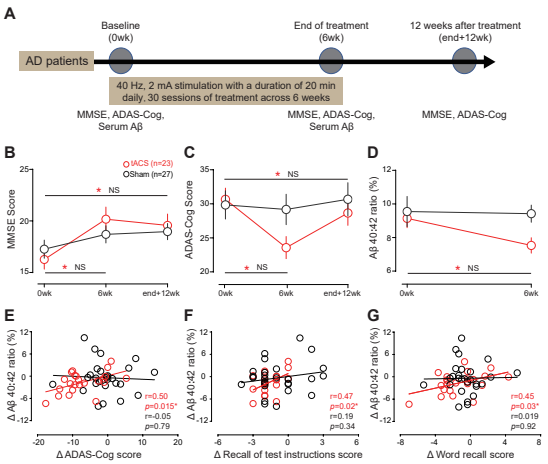

Figure 1 Effects of tACS on cognitive functions and serum Aβ levels in patients with AD. (A) Consolidated Standards of Reporting Trials diagram of the primary phases of the clinical trial. (B,C) MMSE score (B) and ADAS-Cog total score (C) at the designated time points. There was no significant difference between tACS group and sham group at baseline for either MMSE ($F=0.60$, $p=0.44$) or ADAS-Cog total score ($F=0.09$, $p=0.77$). Repeated measures analysis of variance revealed a significant time effect (MMSE: $F_{(2,48)}=33.91$, $p<0.01$; ADAS-Cog: $F_{(2,48)}=10.84$, $p<0.01$) and an interaction effect (time×group) (MMSE: $F_{(2,48)}=5.92$, $p<0.01$; ADAS-Cog: $F_{(2,48)}=6.43$, $p=0.002$), but not a group effect (MMSE: $F_{(1,48)}=0.079$, $p=0.78$; ADAS-Cog: $F_{(1,48)}=0.70$, $p=0.41$) on both MMSE (B) and ADAS-Cog total scores (C). In the tACS group, the MMSE score was significantly higher at the end of ($p_{6wk}=0.012$) or 12 weeks after the treatment ($p_{end+12wk}=0.034$) compared with the baseline; meanwhile, a significant decrease of ADAS-Cog total scores was seen at the end of ($p_{6wk}=0.005$), but not 12 weeks after the treatment ($p_{end+12wk}=0.411$). MMSE (B) and ADAS-Cog total scores (C) remained comparable at any time points in the sham group ($p>0.05$). (D.) after the 6 week treatment, the Aβ 40:42 ratio significantly decreased in tACS group (0 week: 9.14±0.56, 6 week: 7.52±0.46; $t=2.28$, $p=0.03$) but not in sham group (0 week: 9.55±0.90, 6 weeks: 9.39±3.00; $t=0.13$, $p=0.90$). (E–G.) Pearson correlation analyses on the change of Aβ 40:42 ratios and that of ADAS-Cog total scores (E), recall of test instructions scores (F) or word recall scores (G). Statistically significant correlations were found in the tACS group but not in the sham group. The correlation coefficient *r* and the corresponding p value for tACS (in red) and sham (in black) groups are displayed (E–G). Data are shown as mean±SE of mean (B–D). *p<0.05. AD, Alzheimer's disease; ADAS-Cog, Alzheimer's Disease Cognitive Component Assessment; MMSE, Mini-Mental State Examination; NS, not significant; tACS, transcranial alternating current stimulation.

RESULTS

A total of 23 cases in the gamma tACS (real stimulation) group and 27 cases in the sham stimulation group completed the whole trial. There were no significant differences between the tACS group and the sham group with regard to age, sex, duration of disease, education level, MMSE or ADAS-Cog scores (all $p>0.05$) at baseline (online supplemental table S1).

In the tACS group, Bonferroni's post hoc tests revealed that there was a significant difference for both MMSE and ADAS-Cog scores at the end of stimulation (MMSE: $p_{6wk}=0.012$, ADAS-Cog: $p_{6wk}=0.005$) compared with the baseline (figure 1B,C). Interestingly, while the MMSE score remained significantly higher at 12 weeks after the end of the stimulation ($p_{end+12wk}=0.034$) (figure 1B), the ADAS-Cog score essentially returned to the baseline ($p_{end+12wk}=0.411$) (figure 1C).

Further, repeated measures (RM) analysis of covariance (ANCOVA) on 11 domains of ADAS-Cog showed that there was a significant time effect on word recall ($F_{(2,48)}=4.12$, $p=0.019$), recall of test instructions ($F_{(2,48)}=20.96$, $p<0.01$), ideational praxis ($F_{(2,48)}=3.92$, $p=0.023$), together with a significant interaction of group-by-time effect on recall of test instructions ($F_{(2,48)}=9.94$, $p<0.01$) and ideational praxis ($F_{(2,48)}=5.35$, $p=0.006$). The effect of the group was only significant in recall of test instructions ($F_{(1,48)}=9.77$, $p=0.003$) and ideational praxis ($F_{(1,48)}=5.08$, $p=0.029$), but not for word recall ($F_{(1,48)}=0.83$, $p=0.37$).

The results of RM ANCOVA on other individual domains of ADAS-Cog were comparable between two groups. Covariates in the RM ANCOVA analysis included age, sex, duration of disease and education level.

We examined Aβ 40 and Aβ 42 levels, and Aβ 40:42 ratios in the peripheral blood with ELISA. At baseline, there was no significant difference between two groups (all $p>0.05$) (online supplemental table S1). Notably, at the end of stimulation, the ratio of Aβ 40:42 significantly decreased compared with that of the baseline in the real stimulation group ($t=2.28$, $p=0.03$) but not in the sham group ($t=0.13$, $p=0.90$) (figure 1D). Correlation analysis revealed a significant correlation between the change of Aβ 40:42 ratios and that of total ADAS-Cog scores from baseline to week 6 (figure 1E) in the tACS group. Further analysis showed a significant association between the alteration of Aβ 40:42 ratios and that of recall of test instructions or word recall score (figure 1F–G) in the tACS group, after covarying for age, sex, duration of disease and education level.

DISCUSSION

To our knowledge, the present study for the first time investigated the clinical safety and effects of temporal lobe gamma tACS on cognitive function of patients with AD. We demonstrated that 30 sessions of a daily 20 min tACS treatment over 6 weeks significantly improved the cognitive function in patients with mild to moderate AD, accompanying peripheral changes in blood Aβ 40:42 ratios. These results support the potential role of gamma oscillation as a therapeutic strategy for AD.

Temporal lobe tACS directly affects entorhinal cortices and hippocampal network, which are important for cognitive functions. Gamma frequency oscillation is generated by interneuron networks and is critical for temporally binding network information in the brain, which contributes to memory formation and sensory processing.[2] It is also found that gamma tACS enhances long-term potentiation (LTP)-like plasticity induced by intermittent theta-burst stimulation when applied to the primary motor cortex or M1, whose effects are correlated to short-interval intracortical inhibition changes, a neurophysiological biomarker for γ-aminobutyric acid (GABA)-A receptor activities.[3] This suggests that gamma tACS might directly modulate GABAergic transmission in the cortical regions with potential lasting effects (eg, continuous increase at gamma band) and reverse the interneuron (eg, fast-spiking interneuron) dysfunctions in AD.[4] Additionally, gamma tACS might result in oscillation changes at other bands (eg, beta)[5] and affect other aspects of information processing, which all contribute to the behavioural improvements.

This study has several limitations. First, the total sample size is relevantly small, and only patients with mild to moderate AD were recruited. Second, it will be interesting to perform brain imaging or electroencephalogram recordings to elucidate the neural network connectivity changes after tACS treatment, both at the resting state and during a cognitive task. Third, peripheral amyloid levels may not timely reflect the central changes (to be detected with the cerebral spinal fluid or PET imaging), and the blood sampling is limited by two time points.

In conclusion, the present study demonstrates the safety and clinical efficiency of gamma tACS on cognitive functions of patients with AD, but the therapeutic effects may not last long after the treatment. Future large-scale clinical trials are warranted to unveil the long-term effects of tACS in AD treatment.

Dongsheng Zhou,[1] Ang Li,[2,3,4,5] Xingxing Li ,[1] Wenhao Zhuang,[1] Yiyao Liang,[2] Cheng-Ying Zheng,[1] Hong Zheng,[1] Ti-Fei Yuan [6,7,8]

[1]Ningbo Kangning Hospital, Ningbo, Zhejiang, People's Republic of China
[2]Guangdong Key Laboratory of Non-human Primate Research, Guangdong-Hong Kong-Macau Institute of CNS Regeneration, Jinan University, Guangzhou, Guangdong, People's Republic of China
[3]Bioland Laboratory (Guangzhou Regenerative Medicine and Health Guangdong Laboratory), Guangzhou, Guangdong, People's Republic of China
[4]Key Laboratory of CNS Regeneration (Jinan University), Ministry of Education, Guangzhou, Guangdong, People's Republic of China
[5]Department of Neurology, Guangdong Neuroscience Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, People's Republic of China
[6]Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China
[7]Co-innovation Center of Neuroregeneration, Nantong University, Nantong, Jiangsu, People's Republic of China
[8]Translational Research Institute of Brain and Brain-Like Intelligence, Shanghai Fourth People's Hospital Affiliated to Tongji University School of Medicine, Shanghai, People's Republic of China

Correspondence to Dr Ti-Fei Yuan, Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, People's Republic of China; ytf0707@126.com

Acknowledgements The authors thank Qi Zhou and Xiaoli Liu for assistance in the data collection.

Contributors DZ and TY designed the study; DZ, AL, XL, WZ, YL, C-YZ and HZ performed the experiments; DZ, AL, XL, WZ and TY analysed the results and wrote the paper. All authors read and approved the final version of the manuscript.

Funding Supported by grants from the Basic Public Welfare Project of Zhejiang Province (LGF21H090008), Ningbo Natural Science Foundation (2019A610297) to DZ; Natural Science Foundation of Zhejiang Province (LQ21H170001), Zhejiang Medical and Health Science and Technology programme (2020KY285 and 2020KY286) to XL and WZ; Science and Technology Commission of Shanghai Municipality (18JC1420304) to TFY; NSFC (82071372), Guangdong Key Laboratory of Non-human Primate Research (2020B121201006), Outstanding Scholar Program of Bioland Laboratory (2018GZR110102002), Science and Technology Program of Guangzhou (202007030012) to AL.

Competing interests None declared.

Patient consent for publication Consent obtained from parent(s)/guardian(s)

Ethics approval The procedure was in compliance with the ethical standards and regulations of human studies of the Declaration of Helsink. The trial was registered at China Registry of Clinical Trials (ChiCTR1800018370).

Provenance and peer review Not commissioned; externally peer reviewed.

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jnnp-2021-326885).

DZ, AL, XL and WZ contributed equally.

To cite Zhou D, Li A, Li X, *et al*. *J Neurol Neurosurg Psychiatry* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jnnp-2021-326885

Received 15 April 2021
Accepted 20 October 2021

J Neurol Neurosurg Psychiatry 2021;**0**:1–3. doi:10.1136/jnnp-2021-326885

ORCID iDs
Xingxing Li http://orcid.org/0000-0003-3929-7625
Ti-Fei Yuan http://orcid.org/0000-0003-0510-715X

REFERENCES

1 Etter G, van der Veldt S, Manseau F, *et al*. Optogenetic gamma stimulation rescues memory impairments in an Alzheimer's disease mouse model. *Nat Commun* 2019;10:5322.
2 Iaccarino HF, Singer AC, Martorell AJ, *et al*. Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature* 2016;540:230–5.

3 Guerra A, Suppa A, Bologna M, *et al*. Boosting the LTP-like plasticity effect of intermittent theta-burst stimulation using gamma transcranial alternating current stimulation. *Brain Stimul* 2018;11:734–42.

4 Bartos M, Vida I, Jonas P. Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat Rev Neurosci* 2007;8:45–56.

5 Mastakouri AA, Scholkopf B, Grosse-Wentrup M. Beta power may meditate the effect of Gamma-TACS on motor performance. *Annu Int Conf IEEE Eng Med Biol Soc* 2019;2019:5902–8.

Supplemental Table S1. Demographic and clinical characteristics of the AD patients

	tACS (n=23)	Sham (n=27)	t /χ^2	*p* value
Age (years)	76.95±1.28	75.78±1.26	0.65	0.52
Gender			0.97	0.32
Male	8 (34.8%)	6 (22.2%)		
Female	15 (65.2%)	21 (77.3%)		
Disease Duration (years)	4.32±0.38	4.12±0.28	0.43	0.67
Education			0.73	0.69
Illiteracy	3 (13.0%)	6 (22.3%)		
Junior	10 (43.5%)	11 (40.7%)		
Senior	10 (43.5%)	10 (37.0%)		
MMSE	16.26±0.94	17.26±0.88	0.77	0.44
ADAS-Cog	30.65±1.66	29.84±2.08	0.30	0.77
Aβ 40 (pg/ml)	176.51±10.91	187.89±18.69	0.50	0.62
Aβ 42 (pg/ml)	19.30±0.21	19.66±0.25	1.18	0.29
Aβ 40/42 ratio	9.14±0.56	9.55±0.90	0.37	0.71

Data are shown as Mean ± Standard Error of Mean (SEM). Continuous variables were inspected for normality by Kolmogorov-Smirnov test and then compared by Student's *t*-test, and categorical variables by a chi-square test. AD: Alzheimer's disease; MMSE: Mini Mental State Examination; ADAS-Cog: Alzheimer's Disease Assessment-Cognitive Component.