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Neuropsychological, Biological, and Electrophysiological Outcomes of Gamma-tACS in MCI-AD: A Case Series

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Abstract

Gamma-frequency transcranial alternating current stimulation (gamma-tACS) is a promising neuromodulatory approach for Alzheimer's disease (AD), given the central role of gamma-band dysfunctions in AD-related neuropathology and cognitive decline. Although multi-session gamma-tACS has shown beneficial cognitive and electrophysiological effects in AD, its impact in Mild Cognitive Impairment due to AD (MCI-AD), particularly on blood-based biomarkers and gamma entrainment, remains poorly understood. This case series provides preliminary evidence on the feasibility, tolerability, and multidimensional effects of 40 Hz tACS in individuals with biomarker-confirmed MCI-AD. Three participants completed 20 sessions of bilateral temporal gamma-tACS (40 Hz, 2 mA, 60 minutes/day, 5 days/week for 4 weeks). Assessments were conducted at baseline, post-intervention, and at 3-month follow-up. Outcome measures included neuropsychological testing, functional scales, blood biomarkers, resting-state electroencephalography (EEG), and 40 Hz auditory entrainment. Change magnitudes were descriptively estimated using within-subject Hedges' g. Gamma-tACS was highly feasible and well tolerated, with high adherence and only mild transient side effects. Cognitive outcomes showed overall positive trends, including improvements in global cognition and delayed associative memory. Functional outcomes (i.e., autonomies, cognitive complaints and affective evaluation) were mixed, while blood biomarkers had heterogeneous changes, except for a consistent reduction in the Aβ42/Aβ40 ratio. EEG analyses revealed participant-specific modulations and enhanced offline gamma entrainment. Despite limitations related to sample size and study design, these findings support further investigation of gamma-tACS as an early intervention targeting oscillatory dysfunctions AD-MCI.

1 INTRODUCTION

Dementia encompasses a range of neurodegenerative disorders characterized by progressive cognitive decline, along with alterations in mood and behavior, that significantly impair daily functioning. According to recent estimates from the Global Burden of Disease Study, approximately 57 million individuals were living with dementia globally in 2019, a number projected to nearly triple to 153 million by 2050 due to population growth and aging (Nichols et al. 2022). Alzheimer's disease (AD) is the most prevalent cause of dementia (Alzheimer's Association 2024). AD-associated cognitive decline typically manifests through episodic memory impairment, and involves a neuropathological cascade of microglia dysfunctions, accumulation of extracellular β-amyloid (Aβ) plaques, and intracellular neurofibrillary tangles of phosphorylated tau protein (p-tau) in the brain (Sheppard and Coleman 2020). These alterations lead to imbalanced neuronal activity, reduced neuronal synchrony, and disrupted oscillatory activity at local and network levels (Affoo et al. 2013; Manippa et al. 2024b).

In recent years the need for reliable biomarkers for neurodegenerative diseases (Jack et al. 2013) has significantly advanced interest in the preclinical stages of AD (Dubois et al., 2016). AD is now understood as a dynamic, multidimensional continuum that evolves from a preclinical phase, characterized by cognitively intact individuals exhibiting biochemical indicators of AD, to clinically apparent stages (Jack et al. 2013; Jack Jr. et al. 2024). A critical transitional phase, known as Mild Cognitive Impairment (MCI), bridges normal aging and dementia, offering a potentially important therapeutic window for early intervention. Cognitive decline in MCI exceeds age- and education- related expectations, yet does not substantially interfere with daily functioning or autonomy (Winblad et al. 2004; Gauthier et al. 2006). The classification of MCI can be based on specific cognitive deficits (e.g. amnesic or non-amnesic; Petersen, 2004) or its etiopathology (e.g., MCI-AD; (Albert et al. 2011). Epidemiological data indicate that approximately 10–15% of individuals with MCI progress to dementia within one year (Cooper et al. 2015), and up to 60% convert within five years (Gauthier et al. 2006). Hence, this transitional stage offers an optimal window for therapeutic intervention to effectively influence disease progression. Although several pharmacological treatments have been developed, currently only anti-amyloid therapies are specifically indicated for MCI. Moreover, while anti-amyloid antibodies can slow amyloid-related pathology, their measurable effects on cognitive decline remain modest, and available interventions still fail to address the broader neurocognitive deterioration driven by AD's complex and multifactorial mechanisms. (Knopman et al. 2021; Logroscino et al. 2025).

A promising perspective in this field involves the use of non-invasive brain stimulation (NIBS) that modulates brain oscillations and the coordinated activity of neural ensembles. Notably, gamma waves are of great interest here due to their key role in information processing, higher cognitive functions, and the underlying cross-frequency synchronization (Başar 2013; Grent-'t-Jong et al. 2018; Adaikkan et al. 2019). Moreover, they appear to be critically involved in hippocampal-dependent memory functions (Kirwan and Stark 2004; Manippa et al. 2025b), processes among the earliest to be affected by AD neuropathology (Hampel et al. 2008; Scianatico et al. 2024). Coherently, there is substantial evidence of gamma-band (> 30 Hz) disruption in AD patients (Jafari et al. 2020; Güntekin et al. 2023), which origin from the degeneration of GABAergic interneurons and cholinergic projections, driven by pathological accumulation of extracellular Aβ and tau-related neurofibrillary tangles (Stam et al. 2002; Palop et al. 2007; Mably and Colgin 2018).

Building on this mechanistic insight, gamma oscillations have emerged as a promising therapeutic target. One approach to modulate gamma activity is through entrainment, a process in which exogenous stimulation (sensory or electrical) delivered at a specific frequency is used to synchronize endogenous neural oscillations (Elyamany et al. 2021; Menardi et al. 2022). For instance, 40Hz stimulation delivered via optogenetics as well as visual and/or auditory stimulation in animal models of AD have been shown to alleviate cognitive symptoms and reduce key pathophysiological markers, including Aβ and p-tau (Iaccarino et al. 2016; Manippa et al. 2022). Although some clinical trials are currently exploring gamma entrainment using sensory stimulation (Chan et al. 2022), evidence remains limited (Manippa 2025), whereas a more extensive body of literature exists on the effects of gamma-frequency transcranial alternating current stimulation (tACS). TACS applies a low-intensity sinusoidal electrical current to the brain through surface electrodes placed over the scalp (Wischnewski et al. 2023). A recent systematic review examined the use of gamma-tACS in patients with MCI and AD, highlighting its safety, tolerability, and therapeutic potential (Manippa et al. 2024b). Across 6 multi-session studies, most patients reported cognitive improvements, particularly in memory functions, and increase in resting state EEG gamma activity (Zhou et al. 2022; Tang et al. 2024; Cantoni et al. 2025). Inconsistent findings, instead, emerged from neuropathological markers such as Aβ and p-tau analyses (Dhaynaut et al. 2022; Zhou et al. 2022; Cantoni et al. 2025).

Therefore, gamma-tACS has already been demonstrated to be a feasible and effective method for entraining gamma oscillations and improving cognitive functions in patients with AD (Manippa et al. 2024b; De Paolis et al. 2024). Nevertheless, studies involving individuals on the MCI-AD continuum remain

limited. Furthermore, there is a notable gap in the investigation of the effect of gamma tACS on less invasive, and cost-effective biomarkers, such as blood-based ones, which could provide valuable insights into treatment mechanisms and disease progression. Similarly, research on the effects of gamma-tACS on neuroplasticity, which might be involved in entrainment, is still scarce, leaving open questions about its long-term impact on brain oscillation and network reorganization. To address these gaps, we initially designed a randomized, double-blind, placebo-controlled trial to assess the effects of multi-session 40 Hz-tACS in individuals with MCI-AD on neuropsychological and functional outcomes, blood biomarkers, and neurophysiological measures. However, due to recruitment issues causing early termination of the trial, only three participants received active stimulation. Although limited in size and lacking a control group, this case series offers a preliminary, in-depth exploration of both functional and biological responses to gamma-tACS in MCI patients, providing early insight into its potential mechanisms of action and clinical relevance in the context of prodromal AD.

2 METHODS

2.1 Study design

This study was originally conceived as a single-center, double-blind, randomized controlled trial conducted at the *Center for Neurodegenerative Diseases and the Aging Brain*, University of Bari "Aldo Moro" at Pia Fondazione "Cardinale G. Panico", Tricase, Lecce, Italy. Ethical approval was obtained from the regional ethics committee (Azienda Sanitaria Locale Lecce (ASL LE), Verbal N◦11, 11th January 2023, protocol number: 231412/ 2022). However, due to early termination of the trial due to difficulties in recruiting participants, largely related to the logistical commitment for patients, only four participants were enrolled. One participant allocated to the sham condition withdrew before baseline assessments due to an acute exacerbation of a pre-existing medical condition unrelated to the study intervention. The remaining three participants, all of whom received active stimulation, completed the study protocol. Thus, the present report includes data of these three patients, who blindly received active gamma-tACS. Outcome measures included cognitive and functional (i.e., autonomies, cognitive complaints and affective status) evaluation, EEG, and blood-based biomarkers, assessed at baseline (T0), post-intervention (T1), and three months after protocol completion (T2). The intervention protocol and outcome measures are summarized in Figure 1.

2.2 Participants

Four patients (2 female and 2 male, age range 63-74 years old, education level ranges 8-13 years) were recruited according to the National Institute on Aging and the Alzheimer's Association (NIA-AA) criteria for MCI-AD (Albert et al. 2011), which include: i) cognitive decline or change reported by the patient, caregiver, or clinician; ii) objective evidence of impaired performance in one or more cognitive domains, primarily involving memory; iii) preserved independence in instrumental activities of daily living and personal autonomy; iv) non-fulfillment of diagnostic criteria for dementia; v) absence of vascular, traumatic, or other medical conditions explaining the cognitive decline; vi) presence of specific cerebro-spinal fluid biomarkers (Aβ42, t-tau, p-tau181). Structural magnetic resonance imaging (MRI) was performed to assess brain atrophy patterns and exclude other potential neurological causes of cognitive impairment. Exclusion criteria were: i) current or past cerebrovascular disease, hydrocephalus, or intracranial mass present in magnetic resonance imaging (MRI); ii) history of traumatic brain injury; iii) severe medical or psychiatric conditions; iv) history of epilepsy; v) pregnancy or breastfeeding; vi) presence of metal/electronic implants (e.g., pacemakers); vii) age > 75 years; viii) any other condition deemed to make the patients unsuitable for study participation by the investigator.

All participants were literate in Italian, had at least five years of formal education, and were on a stable pharmacological regimen for at least six consecutive weeks prior to the intervention. Particularly, only Pt#3 took cholinesterase inhibitors (i.e., memantine 20mg daily), with no planned dose adjustments during the study, while other participants were not taking any AD-specific medication. Written informed consent was obtained from all participants. Baseline demographic, biomarker, and neuropsychological screening results for diagnosis are summarized in Table 1.

Table 1. Descriptives of demographics, cerebrospinal fluid biomarkers (CSF), MRI visual ratings, and neuropsychological screening for each participant

				CSF Biomarker (pg/mL)			**MRI Visual Rating Scales**				**Neuropsychological Screening**				
Patient	Age (Y)	Edu (Y)	Sex	Aβ42	t-TAU	p-TAU	GCA	MTA	Fazekas	Koedam	CRIq	MMSE	FAB	NPI	bADL
#1	74	8	M	456	752	122.1	1	2	2	2	103	24	16	8	6
#2	65	13	F	557	789	117	1	3	0	0	95	23	10	8	6
#3	63	13	F	617	1080	180.9	1	2	0	1	90	21	14	16	6
#4	68	13	M	597	376	62.5	1	1	1	0	115	29	17	13	6

Note: MRI visual rating scales include: Global Cortical Atrophy (GCA; range 0 – 3), Medial Temporal Atrophy (MTA range 0 – 4), Fazekas scale (range 0 – 3), and Koedam scale (range 0 – 3). For all imaging scales, higher scores indicate greater severity of atrophy or white matter damage. Cognitive Reserve Index Questionnaire (CRIq) scores are normally distributed (M = 100, SD = 15) with higher scores reflecting greater cognitive reserve. Neuropsychological measures include: Mini Mental State Examination (MMSE; range 0 – 30) and Frontal Assessment Battery (FAB; range 0 – 18), where higher scores indicate better cognitive performance; Neuropsychiatric Inventory (NPI; range 0 – 144), where higher scores indicate greater neuropsychiatric symptom burden. Functional autonomy measures are Basic Activities of Daily Living (bADL; range 0 – 6) and Instrumental Activities of Daily Living (iADL; range 0 – 8), with higher scores indicating greater functional independence.

2.3 Intervention

Patients underwent 20 sessions (5 days/week for 4 weeks) of antiphase gamma-tACS delivered via a battery-driven current stimulator (*Brainstim*, EMS, Italy) using a pair of rubber electrodes (4 × 4 cm) positioned bilaterally over the temporal lobes (T3, T4 according to the 10–20 EEG system) for 60 minutes. A conductive Ten20 paste layer was applied to the electrode-scalp interface to ensure optimal conductivity and reduce contact impedance (< 5 kΩ in all sessions). Electrodes were secured to maintain stable placement throughout stimulation. Each session delivered an alternating sinusoidal current of 2.0 mA peak-to-peak (current density: 0.0625 mA/cm²) at 40 Hz for 60 minutes. During each tACS session, participants sat in a quiet, well-lit room, eyes open, avoiding speaking or significant movements. To promote comfort and maintain a consistent brain state, participants watched pre-selected naturalistic documentaries ("Wild Italy", freely available at raiplay.it), minimizing distractions during the tACS sessions. Videos were chosen for their emotionally neutral content, suitable length, and accessible language. Subjective sensations were recorded at the end of each session.

2.4 Outcome measures

Each outcome was measured 1–3 days before treatment initiation (baseline, T0), 1–3 days after the intervention concluded (T1), and 3 months (± 3 days) after treatment ended (T2). This multimodal approach enabled the evaluation of changes in cognition, neural oscillations, and biochemical signatures, providing complementary insights into the potential mechanisms of action and durability of the stimulation effects. All outcome assessments were conducted by trained, blinded clinicians. Crucially, to ensure methodological consistency, each participant was evaluated by the same clinician across all time points.

2.4.1 Feasibility and tolerability

- Feasibility was assessed by recording the number of stimulation sessions completed by each participant relative to the total number planned in the protocol.

- Tolerability (i.e., tACS sessions side effects) was evaluated through a standardized self-report questionnaire administered immediately after each tACS session (Manippa et al. 2024a). This instrument was designed to monitor the occurrence and severity of potential side effects by assessing five predefined categories: cutaneous sensations, phosphenes, fatigue, pain, headache, and confusional states. Participants rated each item using a 5-point Likert scale (0 = absent, 1 = mild, 2 = moderate, 3 = major, 4 = strong), allowing for a graded assessment of symptom intensity. The questionnaire was completed independently by each participant and served as a systematic tool to track tolerability across sessions and individuals.

2.4.2 Cognitive assessment

- The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; (Randolph et al. 1998)) is a standardized neuropsychological battery comprising a total score of global cognition and five sub-scores (i.e., indices): Immediate Memory, Visuospatial/Constructional skills, Language, Attention, and Delayed Memory. RBANS is particularly sensitive to early memory impairment, making it a suitable tool to detect subtle cognitive changes, especially in MCI-AD. Its repeatable design, utilizing alternate parellel forms (Forms A, B and C) to minimize practice effects, ensure reliable design allows for pre- and post- intervention assessments.

- The Digit Span Backward test is a subtest of the Wechsler Adult Intelligence Scale (WAIS) that measures working memory and attention. In this task, the examiner reads a sequence of numbers, and the participant must repeat them in reverse order. The sequences increase in length as the test progresses, making the task progressively more difficult. This measure is useful for evaluating cognitive functions related to short-term memory and executive functioning. The score reflects the longest sequence length that the participant can correctly recall in reverse.

- The Italian Face-Name Associative Test (ItFNAT, (Manippa et al. 2025a, c)) is a neuropsychological test designed to assess cross-modal associative memory, specifically the ability to link faces with proper names, a cognitive function often impaired from the early stages of AD. The ItFNAT is available in three parallel versions, allowing repeated assessments over time (e.g., pre-, post- intervention, and follow-up) while minimizing practice effects. The test provides three scores reflecting different aspects of associative memory performance: Immediate Recall (IR; correct recall of face-name pairs immediately after learning), Delayed Free Recall (DFR; free face-name recall after a 15-minute delay, reflecting memory consolidation), and Delayed Total Recall (DTR; including both DFR and recognition, capturing the combined ability to retrieve and recognize learned associations). Together, these scores allow a detailed evaluation of learning, retention, and retrieval of associative memory, making the ItFNAT a sensitive outcome for detecting cognitive changes following interventions such as neuromodulation.

2.4.3 Functional assessment

- The Hospital Anxiety and Depression Scale (HADS (Zigmond and Snaith 1983)) is commonly used to determine the levels of anxiety and depression experienced in the prior two weeks. The HADS is a fourteen-item scale that generates scores for the two subscales anxiety (7 items) and depression (7 items).

- The Prospective and Retrospective Memory Questionnaire (PRMQ; (Smith et al. 2000)) is a self-report measure designed to assess everyday memory failures. It includes items targeting both prospective memory (e.g., remembering to carry out intended actions in the future, such as taking medication or attending appointments), and retrospective memory (e.g., recalling past information or events, such as names or details of conversations). The PRMQ was completed independently by both patients and caregivers.

- Basic Activities of Daily Living (bADL) and Instrumental Activities of Daily Living (iADL) were used to assess abilities related to self-maintenance and independent living, encompassing self-care, management of the living environment, and acquisition of essential resources (Katz 1963; Lawton and Brody 1969). A reliable identification of such deficits is necessary to distinguish MCI from dementia stage. A higher score describes more preserved autonomy.

2.4.4 Blood-based biomarkers

Peripheral blood samples were collected to quantify plasma AD-related biomarkers, including amyloid-β and phosphorylated tau isoforms, together with Neurofilament Light Chain (NfL), a sensitive biomarker of axonal injury, neuroinflammatory processes, and altered neuroplasticity, using ultrasensitive immunoassays. Blood samples were collected in EDTA tubes and centrifuged for 5 minutes at 2000 rpm at room temperature, within one hour after collection. After centrifugation, plasma was removed, aliquoted (0.5 ml/aliquot) into screw-cap polypropylene tubes and stored at -80 °C until biochemical analyses were conducted. Samples were thawed at room temperature only once before analysis. All plasma samples were analyzed for Aβ42, Aβ40, pTau181, pTau217, and NfL using the Lumipulse fully automated platform G600II, according to the manufacturer's instructions, and commercially available kits (Fujirebio Europe, Ghent, Belgium). For the interpretation of the blood biomarker results, the following cutoff values base on the literature were considered: Aβ42 > 25.1 ± 3.5 (SD) pg/mL, Aβ40 < 291 ± 41 (SD) pg/mL, Aβ42/Aβ40 > 0.086 ± 0.007 (SD) pg/mL, pTau181 < 1.72 ± 0.43 (SD) pg/mL, pTau217 < 0.13 ± 0.11 (SD) pg/mL, NfL < 10 ± 7 (SD) pg/mL (Vermunt et al. 2022; Arranz et al. 2023).

2.5.5 Electroencephalography

EEG was recorded using an EB Neuro system with 19 channels arranged according to the international 10–20 system. The reference and ground electrodes were positioned along the midline of the EEG cap. Signals were sampled at 256 Hz, and electrode impedances were kept below 10 kΩ. EEG data were acquired via Galileo software and pre-processed offline in Matlab 2025b (MathWorks Inc., USA) with the EEGLAB toolbox (v.25.1.0) via custom-written Matlab scripts. Signals were filtered (1–80 Hz band-pass; 50 Hz notch) and visually inspected. Artifact Subspace Reconstruction (Chang et al. 2020) was applied with a burst criterion of 20, a window criterion of 0.25, a channel criterion of 0.85, and a flatline criterion of 5s. Independent component analysis (ICA) was then performed, and components reflecting artifacts (muscle, eye, heart, channel noise, line noise) were removed. Bad channels previously identified were interpolated after ICA, and data were finally re-referenced to the common average. After rejection, an average of 160.1s (SD = 63.7s; mean data loss ≈ 11.7 %) of clean data per session remained. After ICA, an average of 3.8 channels (SD = 1.25) were interpolated, and 0.14 ICA components (SD = 0.35) were removed per recording. Spectral segmentation was conducted in Brainstorm (Tadel et al. 2011) using Welch's method with 1s windows, 50% overlap, and FFT.

- RsEEG Power Spectrum Density: resting state EEG (rsEEG) was acquired in eyes-closed (EC) and eyes-open (EO) conditions. The recording session lasted for a total of 5 minutes, split into two phases: 150 s with eyes open and 150 s with eyes closed. During the whole EEG recording participants were asked to avoid any movement or excessive muscle tension; in the EO phase, participants were instructed to fixate on a central point on a blank screen to minimize eye movement artifacts, while the instruction for the EC phase was to remain relaxed with their eyes closed. Analyses focused on power spectrum density (PSD in signal units^2/Hz) changes. Spectral decomposition was performed across conventional frequency ranges, including delta (1–3 Hz), theta (4–7 Hz), alpha (8–12 Hz), beta (13–29 Hz), slow-gamma (30–49 Hz), fast-gamma (51–80 Hz), and a narrow gamma band centered on the stimulation frequency (38–42 Hz).

- 40 Hz auditory stimulation offline entrainment: the entrainability of narrow-band gamma activity (38–42 Hz) was evaluated as a potential neurophysiological marker of brain plasticity and adaptability to external rhythmic stimulation. First, 150 s EC baseline rsEEG was recorded to establish pre-stimulation activity. Next, participants received 300s of 40 Hz rhythmic auditory stimulation (40Hz-rAS) through over-ear headphones at 75 dB, keeping their eyes closed throughout. Finally, a second 150s EC rsEEG was recorded immediately after stimulation to capture 40Hz-rAS offline effects. Offline entrainment was quantified as the magnitude of post-stimulation narrow-band gamma activity relative to the baseline, capturing the brain's ability to synchronize with external rhythmic input.

3. RESULTS

All outcomes assessed across the three time points for each patient are reported in the Supplementary Material table S1.

3.1 Feasibility and tolerability

All patients except the #2, completed the study and tolerated the intervention well. Pt#2 attended four consecutive sessions, missed one, completed another four, missed one more, and then completed three additional sessions before withdrawing from the study due to unrelated medical condition (i.e., spastic colitis). Among the remaining three patients who completed treatment, overall adherence to scheduled visits reached 98% (59 out of 60 daily tACS sessions; only one session was missed by Pt#3), reflecting excellent compliance despite the demanding schedule of 26 hospital visits, including intervention sessions and pre/post-assessment appointments.

Across all patients, side effects being minimal and short-lived. The most frequently reported sensations were mild cutaneous effects or occasional headaches, though their average intensity remained well below 1 on the 5-point scale. Reports of phosphenes, fatigue, or transient mental fog were infrequent and predominantly rated as absent or mild in intensity. No seizures or signs of epileptiform activity were observed during clinical monitoring or in post-intervention EEG recordings.

Table 2. Distribution of side-effect intensities (percentage of sessions) across 59 completed stimulation sessions. *Ratings reflect patient responses on the standardized 5-point tolerability scale.*

	Reported frequency (%)				
Side Effects	**Absent**	**Mild**	**Moderate**	**Major**	**Strong**
Cutaneous sensations	86.44	10.17	3.39	0.00	0.00
Phosphenes	93.22	5.08	1.69	0.00	0.00
Fatigue	96.61	3.39	0.00	0.00	0.00
Pain	98.31	1.69	0.00	0.00	0.00
Headache	94.92	5.08	0.00	0.00	0.00
Confusional state	96.61	3.39	0.00	0.00	0.00

Notes: Score ranged between 0 ("Absent") and 4 ("Strong")

3.2 Cognitive, Functional Assessment and Blood Biomarkers

Due to the very small sample size, outcome changes across the three time points were explored using within-subject effect sizes corrected for small-sample bias (Hedges' g) as a descriptive measure of change magnitude. Effect sizes were computed from within-subject Cohen's *d* for the key contrasts (T1–T0, T2–T0) and subsequently adjusted using Hedges' correction (g = 0.57). Effect size estimates were calculated to summarize observed within-individual changes and were interpreted in conjunction with patient-by-patient descriptions and visual inspection of individual trajectories. Given the instability of variance estimates resulting from the extremely small sample size, effect sizes were reported for descriptive purposes only and without inferential intent. Values in the moderate-to-large range ($|g| \gtrsim 0.50$) were interpreted as suggestive of potentially meaningful group-level change.

Concerning the RBANS indices, immediate memory, language, and delayed memory indices showed only small to moderate changes between the time points. For the visuospatial/constructional index, tACS induced a moderate improvement at T1 that increased at the follow-up (g = 0.59). Positive effects of tACS were also observed for the attention index, with a strong effect at T1 (g = 0.62), which remained moderate at T2. At the individual level, heterogeneous patterns were observed across RBANS indices. Beyond the visuospatial and attention improvements, Pt#4 showed a marked increase in the attention index from T0 to T1 that was maintained at T2, contributing to a gradual improvement in the total RBANS score over time. Pt#1 exhibited a transient decline in language performance despite overall stability or improvement in other domains, whereas Pt#3 remained largely stable across indices, with only minimal fluctuations over time. These patient-specific trajectories underscore the variability of cognitive responses to tACS in this small case series. Finally, the total RBANS score improved after tACS treatment at both T1 (g = 1.82) and T2 (g = 0.65). Particularly, Pt#1 showed a rapid improvement by T1 that was maintained at T2, Pt#3 remained stable across the time points with a small improvement at T1, whereas Pt#4 demonstrated a gradual increase over time.

Scores on Digit-Span backward and ItFNAT IR and DFR remained largely stable over time points, with no variance for some measures, making effect size calculation inapplicable. On the other hand, the ItFNAT DTR score showed a moderate improvement between T0 and T1, which further increased at the follow-up (g = 1.32). Examining intra-individual trajectories, Pt#1 showed a notable increase from 4 at T0 to 8 at T2, while Pt#3 showed a smaller but consistent improvement over time (from 4 to 6). Even Pt#4 exhibited a substantial gain from 8 at T0 to 10 at T2.

Regarding functional assessments, depression (HADS) and iADL scores showed only small to moderate changes across time points, with no variance for some measures, making effect size calculation inapplicable. In contrast, anxiety symptoms appeared to worsen (i.e., higher scores) after treatment (g = 0.57) and then moderately improved at the follow-up, mainly driven by Pt#1 and Pt#3, whereas Pt#4 showed a gradual reduction over time. As for the PRMQ scores, compared with baseline results, both patients and caregivers reported more cognitive complaints (higher scores) after tACS treatment (all $g \leq -1.14$). At follow-up, an increase in cognitive complaints was still evident in caregiver-reported PRMQ scores but not in patient-reported. Particularly, Pt#1 showed a partial return toward baseline at follow-up, while Pt#3 and Pt#4 remained higher than baseline. Finally, a decrease in bADL scores was observed between T2 and T0 (g = -0.66), primarily in Pt#3 and Pt#4, whereas Pt#1 remained stable across time points. Group-level changes are reported in Table 4 while the more robust changes are also displayed at the individual level in Figure 2A.

Table 3*. *Longitudinal changes in cognitive and functional outcomes. *Mean scores (standard error) are presented for baseline (T0), after the gamma-tACS intervention (T1), and at the 3-month follow-up (T2). Within-group changes over time are quantified using Hedges' g for intervals T1–T0 and T2–T0.*

Measure	T0 Mean (SE)	T1 Mean (SE)	T2 Mean (SE)	T1-T0 Hedges' g (SE)	T2-T0 Hedges' g (SE)
RBANS					
Immediate Memory	25.67 (5.90)	23.67 (6.64)	22.67 (6.57)	-0.17 (0.21)	-0.38 (0.15)
Visuo-Costructive	30.67 (4.49)	34.33 (1.45)	36.33 (1.45)	0.32 (0.31)	0.59 (0.15)
Language	23.00 (0.58)	21.00 (2.65)	21.33 (3.38)	-0.21 (0.68)	-0.14 (0.63)
Attention	25.33 (10.48)	31.33 (13.59)	31.33 (14.84)	0.62 (0.03)	0.46 (0.00)
Delayed Memory	18.33 (4.84)	16.33 (1.86)	15.00 (2.08)	-0.22 (0.03)	-0.39 (0.08)
Total	123.00 (23.25)	126.67 (23.38)	126.67 (25.08)	1.82 (0.02)	0.65 (0.01)
Digit Span Backward	3.68 (1.20)	4.00 (1.16)	4.00 (1.00)	0.33 (0.10)	0.13 (0.26)
ItFNAT					
Immediate Recall	0.00 (0.00)	0.00 (0.00)	0.67 (1.16)	-	-
Delayed Free Recall	0.00 (0.00)	0.33 (0.58)	0.33 (0.58)	-	-
Delayed Total Recall	5.33 (1.33)	7.33 (0.88)	8.00 (1.16)	0.38 (0.56)	1.32 (0.33)
HADS					
Anxiety	2.33 (1.45)	3.33 (1.33)	1.33 (0.88)	0.57 (0.17)	−0.33 (0.26)
Depression	2.00 (0.00)	2.00 (1.53)	0.67 (0.33)	-	-
PRMQ					
Patients	31.67 (2.33)	40.67 (3.76)	33.00 (1.53)	1.94 (0.16)	0.37 (0.17)
Caregivers	55.00 (6.00)	59.00 (5.03)	62.00 (7.00)	1.14 (0.07)	0.87 (0.18)
Functional Autonomies					
bADL	5.00 (0.58)	5.00 (0.58)	4.33 (0.67)	-	−0.66 (0.22)
iADL	8.00 (0.00)	8.00 (0.00)	8.00 (0.00)	-	-

Note: Repeatable Battery for the Assessment of Neuropsychological Status (RBANS); Italian Face-Name Associative Test (ItFNAT); Hospital Anxiety and Depression Scale (HADS); Prospective and Retrospective Memory Questionnaire (PRMQ); Basic Activities of Daily Living (bADL); Instrumental Activities of Daily Living (iADL).

Concerning the blood-based biomarkers, NfL, Aβ40, Aβ42, p-Tau181, and p-Tau217 showed only small to moderate changes across time points, with effect sizes well below the threshold for clinical relevance. Interestingly, Pt#4 showed a marked Aβ40 increase from 181.14 (T0) to 451.45 (T1), while Pt#1 remained relatively stable and Pt#3 increased gradually, highlighting heterogeneous individual trajectories. Consequently, the Aβ42/Aβ40 ratio showed a marked negative change, with a significant decrease compared with the baseline at both T1 (g = -0.58) and T2 (d = -2.10). Pt#1 declined at T2, Pt#3 showed initial worsening at T1 with partial recovery at T2, and Pt#4 displayed a consistent decline. Group-level changes are reported in Table 4, whereas the more robust changes are also displayed at the individual level in Figure 2B.

Table 4. Longitudinal changes in blood biomarkers*. Mean scores (standard error) are presented for baseline (T0), after the gamma-tACS intervention (T1), and at the 3-month follow-up (T2). Within-group change over time is quantified using Hedges' g for intervals T1–T0 and T2–T0.*

Marker (pg/mL)	T0 Mean (SE)	T1 Mean (SE)	T2 Mean (SE)	T1-T0 Hedges' g (SE)	T2-T0 Hedges' g (SE)
NfL	27.34 (2.76)	28.41 (3.74)	27.48 (2.31)	0.29 (0.08)	0.02 (0.27)
Aβ42	20.05 (2.68)	21.68 (3.04)	20.46 (2.26)	0.10 (0.62)	0.03 (0.62)
Aβ40	240.62 (29.74)	330.82 (61.62)	345.32 (46.70)	0.33 (0.71)	0.46 (0.74)
Aβ42/Aβ40	0.08 (0.00)	0.07 (0.01)	0.06 (0.00)	−0.58 (0.03)	−2.10 (1.33)
pTau181	2.59 (0.57)	3.14 (0.32)	2.46 (0.20)	0.32 (0.42)	−0.06 (0.66)
pTau217	0.73 (0.28)	1.12 (0.50)	0.92 (0.18)	0.39 (0.24)	0.34 (0.25)

3.3 Electroencephalographic activity

To characterize longitudinal changes of oscillatory activity, relative power changes were computed for each conventional frequency band (delta, theta, alpha, beta, slow gamma, fast gamma) within the temporal ROI (comprising the tACS intervention target electrodes T3 and T4 and surrounding electrodes T5, F7, T6, and F8) as percentage differences with respect to baseline (T0). Specifically, band-specific power at T1 and T2 was expressed as the percent change relative to T0 for each participant, and group-level deviations from zero were examined descriptively. Given the very small sample size, statistical tests were

not used for inferential purposes. One-sample *t*-tests against zero are reported for completeness only, whereas within-subject effect sizes corrected for small-sample bias (Hedges' g) were used as the primary index to summarize the magnitude of longitudinal change. Group-level changes are reported in Table 5 while individual temporal spectrums are reported in figure 3A.

Table 5. Group-level changes in temporal ROI resting-state EEG spectral power following gamma-tACS intervention. *Data represents percentage changes from baseline (T0) after the gamma-tACS intervention (T1), and at the 3-month follow-up (T2) for both Eyes-Open and Eyes-Closed conditions. Within-group change is quantified using Hedges'g tests against 0.*

	T1-T0 (%)		**T2-T0 (%)**	
	Mean (SE)	**Hedges' g (SE) vs 0**	**Mean (SE)**	**Hedges' g (SE) vs 0**
Eyes-Open				
Delta	22.88 (9.97)	0.76 (0.45)	59.38 (45.84)	0.43 (0.37)
Theta	16.84 (12.97)	0.43 (0.37)	45.02 (36.51)	0.41 (0.37)
Alpha	14.46 (8.21)	0.58 (0.41)	29.78 (17.02)	0.58 (0.41)
Beta	4.98 (4.02)	0.41 (0.37)	5.33 (3.72)	0.47 (0.38)
s-Gamma	-4.96 (5.19)	−0.31 (0.35)	-10.14 (7.48)	−0.45 (0.38)
f-Gamma	-8.08 (8.20)	−0.33 (0.35)	-18.22 (13.82)	−0.43 (0.38)
Eyes-Closed				
Delta	11.47 (9.27)	0.41 (0.37)	34.96 (27.48)	0.42 (0.37)
Theta	9.26 (9.99)	0.31 (0.35)	35.54 (29.35)	0.40 (0.37)
Alpha	8.28 (6.14)	0.45 (0.38)	24.02 (21.91)	0.36 (0.36)
Beta	5.75 (1.91)	0.99 (0.53)	4.21 (3.30)	0.42 (0.37)
s-Gamma	-7.07 (4.06)	−0.58 (0.41)	-13.06 (11.97)	−0.36 (0.36)
f-Gamma	-13.02 (2.41)	−1.78 (0.80)	-21.38 (14.62)	−0.48 (0.38)

Note: Delta (1–3 Hz), Theta (4–7 Hz), Alpha (8–12 Hz), Beta (13–29 Hz), s-Gamma (30–49 Hz), f-Gamma (51–80 Hz).

In the temporal region, during the EO condition**,** a large increase of delta (22.88%, *g* = 0.76) and alpha power (14.46%, *g* = 0.58) was observed at T1. This effect remained moderately increased at T2 (59.38%, *g* = 0.58). During the EC condition, moderate T1 increases were observed for beta (5.75%, *g* = 0.99) while a strong T1 decrease was observed for slow (-7.07%, *g* = -0.58) and fast gamma at T1 (-13.02%, *g* = -1.78). These changes are primarily driven by the pronounced changes observed in Pt#3. In fact, at the individual level, during the EO condition, Pt#1 showed only small changes across all frequency bands at both T1 and T2, while Pt#4 showed intermediate increase in delta and beta power. In contrast, Pt#3 exhibited the largest magnitude of change, characterized by pronounced increases in low-frequency power (delta, theta) and alpha band, particularly at T2, together with substantial decreases in both slow- and fast-gamma activity. A similar pattern was observed for the EC condition, with Pt#3 again showing large increases in low-frequency activity and marked reductions in gamma power at both post-treatment and follow-up, while Pt#4 and Pt#1 displayed minimal or moderate changes.

Auditory gamma entrainment was quantified as the within-session modulation of narrow-gamma power (38–42 Hz) due to rhythmic auditory stimulation. Specifically, for each time point, changes in gamma power within frontal (Fp1, Fp2, Fz, F3, F4), central (Cz, C3, C4), temporal (F7, F8, T3, T4, T5, T6), and posterior ROIs (Pz, P3, P4, O1, O2) were computed by comparing eyes-closed resting-state narrow-gamma power recorded before and after 5 minutes of 40Hz-rAS within the same EEG session. This contrast reflects the brain's capacity to synchronize ongoing neural activity to an external rhythmic input. Group-level changes are reported in Table 6 while individual entrainment is represented in figure 3A.

At baseline (T0), entrainment effects were small across all regions, indicating a limited gamma synchronization capacity before intervention. At the individual level, Pt#1 showed negligible or slight entrainment across regions. In contrast, Pt #3 and Pt#4 already showed modest positive entrainment, particularly over frontal and posterior areas, highlighting inter-individual variability at baseline. Following tACS treatment (T1), narrow-gamma entrainment increased relevantly, with large effect sizes across all scalp regions (frontal *g* = 0.70; central *g* = 1.23; temporal *g* = 1.16; parietal *g* = 0.90), consistent with a robust enhancement of gamma entrainment. This group-level effect was driven by clear increases in all three patients, with Pt#4 showing the largest absolute entrainment values across regions, particularly in central and temporal ROIs, while Pt#1, exhibiting 40 Hz desynchronization at baseline, demonstrated a clear positive shift that reflects a substantial within-subject gain in synchronization capacity. At follow-up (T2), entrainment effects further increased and were maintained at large magnitudes, especially over central (*g* = 1.82) and temporal regions (*g* = 3.48), with large effects also evident over frontal (*g* = 1.01) and parietal sites (*g* = 1.51). At the individual level, Pt#4 continued to show the strongest entrainment across regions, while Pt#3 demonstrated sustained moderate-to-large entrainment, particularly over frontal and posterior sites. Importantly, Pt#1 maintained positive entrainment at T2 across all regions, despite lower absolute values than the other patients, suggesting a durable improvement relative to baseline rather than a transient effect.

Table 6. Individual and group-level effects of 40 Hz auditory stimulation on offline neural entrainment across cortical ROIs. *Mean scores (SE) and standardized effect sizes represent the magnitude of 40 Hz oscillatory synchronization at baseline (T0), after the gamma-tACS intervention (T1), and at the 3-month follow-up (T2). The within-group change is quantified using Hedges' g tests against 0.*

	T0		T1		T2	
Measure	**Mean (SE)**	**Hedges' g (SE) vs 0**	**Mean (SE)**	**Hedges' g (SE) vs 0**	**Mean (SE)**	**Hedges' g (SE) vs 0**
Frontal ROI	0.018 (0.021)	0.27 (0.35)	0.031 (0.015)	0.70 (0.43)	0.027 (0.009)	1.01 (0.53)
Central ROI	0.007 (0.010)	0.23 (0.34)	0.035 (0.009)	1.23 (0.60)	0.020 (0.004)	1.82 (0.81)
Temporal ROI	0.014 (0.016)	0.29 (0.35)	0.037 (0.011)	1.16 (0.58)	0.024 (0.002)	3.48 (1.46)
Posterior ROI	0.022 (0.017)	0.42 (0.37)	0.023 (0.008)	0.90 (0.49)	0.020 (0.004)	1.51 (0.70)

Discussion

This exploratory case series provides evidence that multi-session gamma-tACS induces measurable cognitive, functional, and neurophysiological changes in individuals with MCI-AD. Although only three participants completed the active intervention, our findings align with the emerging literature on the positive effects of gamma-tACS in AD patients (Manippa et al. 2024b). Adherence was exceptionally high, side effects throughout the sessions were minimal and transient. These results are consistent with previous multi-session studies reporting excellent tolerability of gamma-tACS in MCI and AD (Sprugnoli et al. 2021; Dhaynaut et al. 2022; Zhou et al. 2022; Cantoni et al. 2025), suggesting that high-frequency stimulation is safe, even in clinically fragile populations.

Beyond feasibility, cognitive outcomes revealed selective yet meaningful improvements. The RBANS total score increased substantially after treatment and at follow-up in Pt#1 and Pt#4, driven primarily by gains in attention and visuospatial/constructional abilities. Working memory and immediate and delayed free recall of face-name associations showed modest, and heterogeneous changes, while improvement in DTR was more robust among all patients. These cognitive improvements are consistent with prior reports of cognitive enhancement in AD patients following gamma-tACS interventions (Zhou et al. 2022; Tang et al. 2024; Cantoni et al. 2025) and older healthy adults (Grover et al. 2022). Despite these measurable improvements in objective cognitive measures, corresponding improvements were not observed in subjective and functional outcomes. Anxiety levels showed a transient increase immediately after treatment before partially normalizing at follow-up. In parallel, both patient- and caregiver- reported PRMQ scores indicated a higher frequency of everyday memory failures at post-treatment and follow-up (Balash et al. 2013). These complaints were only partially accompanied by functional decline, with autonomy remaining largely preserved, with the exception of a slight worsening in bADL observed at follow-up in Pt#3 and Pt#4. The PRMQ is difficult to interpret, as it may either over- or under-estimate actual daily difficulties, being strongly influenced by insight and subjective appraisal. This is evident both at the group level, as suggested by discrepancies between PRMQ scores and objective cognitive trajectories, and at the individual level, as illustrated by Pt#3, who displayed the most severe cognitive and functional profile yet reported comparatively better PRMQ scores than the other patients. Similar dissociations have been described in other studies with MCI samples, where it was shown that increased insight or heightened caregiver vigilance can amplify perceived deficits despite stable or even improved objective performance (Edmonds et al. 2014; Milanovic et al. 2023). One plausible interpretation is that tACS-related cognitive enhancement may have increased metacognitive awareness, leading participants to detect and report everyday lapses more readily. This heightened awareness could have also contributed to a temporary increase in anxiety, particularly in the immediate post-treatment phase, as individuals become more conscious of their cognitive vulnerabilities (Wynn et al. 2025). Taken together, these findings suggest that temporal gamma-tACS in MCI-AD may primarily act on early network-level mechanisms supporting cognitive efficiency and memory consolidation, with greater benefits emerging in individuals with milder baseline impairment.

Blood biomarkers showed only modest fluctuations. Most markers, including NfL, Aβ42, pTau181, and pTau217, remained stable, suggesting no measurable effect of gamma-tACS intervention on neurodegeneration or tau pathology within the protocol timeframe. These biomarkers are generally considered trait-like indicators of long-term disease burden in AD, reflecting cumulative amyloid and tau deposition and neurodegeneration over years or decades, rather than state-sensitive markers of rapid neurophysiological changes or short-term network modulation (Milà-Alomà et al. 2025). Accordingly, previous multi-session studies have reported mixed results, with some showing reductions in tau markers or amyloid (Dhaynaut et al., 2022; Zhou et al., 2021), while others have reported no significant biochemical effects (Sprugnoli et al., 2021; Cantoni et al., 2025).

On the other hand, none of the markers showed changes indicative of neuronal injury (i.e., NfL), confirming the biological safety of the intervention protocol. The most consistent change observed was a decrease in the Aβ42/Aβ40 ratio, a pattern typically interpreted as unfavorable. Interestingly, while this reduction is usually driven by a decline in Aβ42, in our participants, it was consistently associated with an increase in Aβ40. It is possible that these changes may not directly reflect an increase in brain amyloid deposition but are instead related to alterations in peripheral amyloid metabolism and clearance, or vascular dynamics that may influence plasma Aβ40 levels independently of central plaque load (Teunissen et al. 2022). Given the small sample size, heterogeneous trajectories, and the known variability of plasma amyloid measures, these findings remain preliminary and should be interpreted cautiously. Peripheral biomarkers capture slow, cumulative pathology, whereas induced neurophysiological plasticity occurs over much shorter periods and may not be reflected by trait-like markers.

In fact, EEG findings provide the clearest mechanistic insight. During EO rsEEG, delta and alpha activity increased within the temporal ROI, primarily between T1 and T0. In contrast, during EC rsEEG, increased spontaneous beta activity and reduced gamma activity were observed between T1 and T0. These patterns were largely driven by the trajectories of Pt#3 and Pt#4. While several studies have reported increases in spontaneous gamma following gamma-tACS interventions (Sprugnoli et al. 2021; Cantoni et al. 2025), the heterogeneity of the present EEG findings and the very small sample size, disease progression and possible habituation to the intervention may have contributed to the observed changes. Hence, these results may reflect mixed and partly patient-specific trajectories, which are difficult to disentangle from natural clinical evolution. This is relevant given the debated role of gamma oscillations in AD, which may reflect both functional network communication and pathological hyperexcitability in AD (Van Deursen et al. 2008; Simfukwe et al. 2025). The rise in delta power, typically associated with disease progression, was accompanied by increases in alpha and beta activity, which are often linked to more organized cortical processing, with alpha enhancement recognized as a marker of more preserved large-scale network integrity (Babiloni et al. 2016;

Ranasinghe et al. 2021). However, given the interindividual variability, this combination should be interpreted cautiously and cannot be unequivocally attributed to compensatory mechanisms.

Crucially, after 40 Hz-rAS we observed an increase in narrow-band gamma activity (38 – 42 Hz) from baseline to post-treatment, with effects still detectable at the 3-month follow-up, most clearly in Pt#1 and Pt#4. While previous studies have suggested that gamma entrainment may reflect network-level plasticity (Naro et al. 2016; Palmisano et al. 2025), the specific mechanisms underlying the present changes cannot be determined from these data. Accordingly, the increased 40 Hz entrainment may be interpreted as a functional change in oscillatory responsiveness to stimulation. The individual trajectories provide additional insight into the heterogeneity of gamma-tACS responses. Pt#1 and Pt#4, reporting mild biomarker burden and relatively preserved cognition at baseline, showed the clearest pattern of benefit, including improvements in RBANS and ItFNAT delayed recall, stable functional performance, and increased gamma entrainability. In contrast, Pt#3, who had the most severe diagnostic profile (including CSF biomarkers, cognitive screening deficits, and neuropsychiatric symptoms) along with atypical baseline electrophysiological activity (EC rsEEG resembling the EO rsEEG pattern), showed minimal cognitive gains, a pronounced shift toward delta activity, and an atypical entrainment response, with the slowest rsEEG spectrum observed across the protocol. In particular, the increase in delta activity cannot be interpreted as either beneficial or detrimental, but rather as a non-specific marker related to the broader clinical and neurophysiological context. These participant-level patterns should be considered exploratory and highlight the need to consider baseline neurophysiological integrity when evaluating responsiveness to gamma neuromodulation.

Taken together, this case series study converges in the concept that multi-session gamma-tACS is not only feasible and well-tolerated in MCI-AD but can also induce measurable neuropsychological and neurophysiological improvements. These effects were most evident in participants with milder baseline impairment and relatively preserved network integrity. The absence of clear biomarker changes and the paradoxical increase in subjective complaints temper the interpretation but do not preclude the possibility that gamma-tACS may selectively support early network-level processes and cognitive efficiency.

Strengths, Limitations, and Future Directions

A major strength of this study is its multimodal design, integrating cognitive testing with parallel versions, functional and metacognitive measures, blood-based biomarkers, and detailed electrophysiological analyses. This comprehensive approach captured dissociations between objective performance, subjective experience, and neural dynamics. The systematic assessment of mood, self-perceived cognitive failures, and caregiver-reported decline represents a methodological innovation rarely implemented in prior gamma-tACS studies. Particularly, the use of cognitive tests with parallel versions (RBANS and ItFNAT) ensured that cognitive improvements cannot be attributed to practice effects-a methodological strength rarely implemented in prior tACS studies. Furthermore, this study is among the first to systematically track mood, self-perceived cognitive failures, and caregiver-perceived decline in the context of gamma-tACS, providing novel insight into how neuromodulation may interact with metacognition and illness awareness. Furthermore, the combined analysis of resting-state EEG and 40Hz entrainment provides one of the first integrated views of how tACS influences both spontaneous and stimulus-locked oscillatory activity.

The study also comes with limitations. The small sample size and absence of a control group preclude causal inferences. The short follow-up period may have been insufficient to detect changes in blood-based biomarkers, which often evolve slowly. The heterogeneity of baseline profiles, particularly the markedly more severe condition of Pt#3 who was the only undergoing cholinesterase inhibitor therapy, further complicates interpretation but also highlights the potential importance of early intervention. Another consideration concerns the variability of stimulation targets and tACS parameters across studies (Manippa et al. 2024b). Gamma-tACS studies vary widely in montage, electrode size, current intensity, and session structure, often stimulating different cortical regions, such as the prefrontal cortex (Sprugnoli et al. 2021), precuneus (Cantoni et al. 2025), or angular gyrus (Cappon et al. 2023), with limited theoretical convergence. This diversity reflects the exploratory stage of the field and highlights the ongoing need to clarify which targets and parameters are most effective. Although our temporal-lobe montage aligns with several recent protocols, cross-study comparisons remain challenging, and future research may benefit from individualized targeting based on anatomical or functional connectivity.

Future studies should prioritize larger randomized controlled designs, longer follow-up intervals, and more frequent biomarker sampling. Stratifying participants based on baseline biomarker burden, cognitive profile, or oscillatory characteristics may help to identify which individuals are most likely to benefit from early neuromodulatory intervention. A crucial next step will also be to clarify which cortical targets are most effective for gamma-tACS and to move toward individualized targeting approaches informed by structural and functional neuroimaging. Personalized montages based on MRI-derived anatomy, functional connectivity, or source-localized gamma deficits may substantially improve current delivery to memory-related networks and enhance treatment responsiveness. Incorporating additional neurophysiological markers, such as connectivity measures, cross-frequency coupling, and source-localized gamma activity, could provide deeper insight into the mechanisms through which tACS exerts its effects. Importantly, recent home-based tACS protocols, administered with caregiver support and targeting regions such as the precuneus or angular gyrus (Cappon et al. 2023; Cantoni et al. 2025), demonstrate that gamma-tACS can be safely delivered outside the laboratory, offering a scalable and ecologically valid model for real-world interventions. Ultimately, integrating electrophysiological, cognitive, and subjective measures will be essential to determine whether gamma-tACS can meaningfully alter the trajectory of prodromal AD.

Declarations

We confirm that all participants provided written informed consent to participate in the study and to the publication of their clinical data as presented in the manuscript. As all participants were competent adults, no legal guardian or proxy consent was required.

Conflict of Interest

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

Consent statement

Ethical approval was obtained from the regional ethics committee (Azienda Sanitaria Locale Lecce (ASL LE), Verbal N◦11, 11th January 2023, protocol number: 231412/ 2022), and written informed consent was secured from all participants or their legal representatives. The study adhered to the principles outlined in the Declaration of Helsinki. Data confidentiality was maintained through anonymization and secure data storage.

CRediT

VM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project Administration, Supervision, Visualization, Writing – original draft; DV: Conceptualization; Data curation, Methodology, Supervision, Writing – review & editing; GFS: Data curation; Formal Analysis; Visualization, Writing – review & editing; GV, AV, CZ: Data curation, Investigation, Writing – review & editing; GS: Data curation, Formal Analysis, Writing – original draft; DU: Conceptualization, Project Administration, Supervision; MAN, PT: Writing – review & editing; DR & GL: Conceptualization, Funding Acquisition, Resources, Supervision.

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Availability of Data and Materials

All data supporting the findings of this study are available within the article and its supplementary materials

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Figures

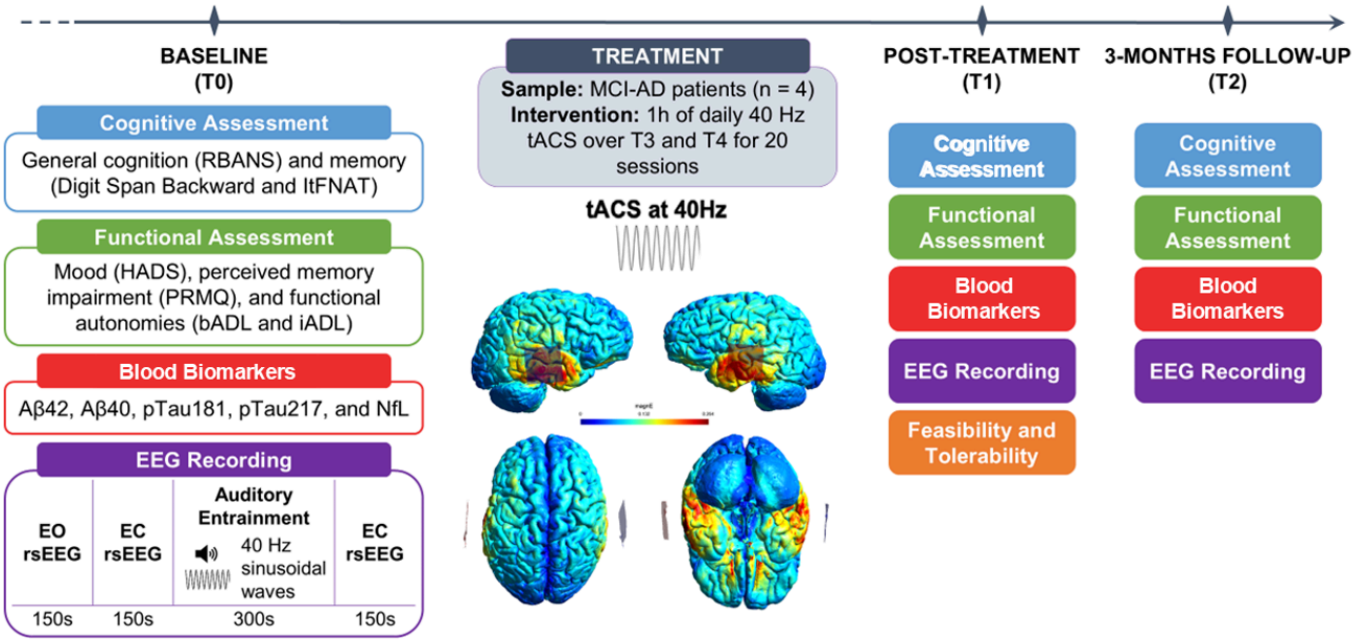


Figure 1

Representation of the study design and assessments. Patients underwent a baseline evaluation (T0), followed by a 20-sessions 40Hz transcranial alternating current stimulation (tACS) intervention, a post-treatment assessment (T1), and a 3-month follow-up evaluation (T2). At baseline (T0), assessments included cognitive evaluation, functional and behavioral measures, blood biomarkers, and EEG recording. Resting-state EEG was acquired with eyes open (EO) and eyes closed (EC), followed by a 40 Hz sinusoidal stimulation auditory entrainment paradigm. T1 and T2 assessments included cognitive and functional evaluations, blood biomarkers, EEG recording and entrainment paradigm. T1 also included evaluation of feasibility and tolerability of the intervention.

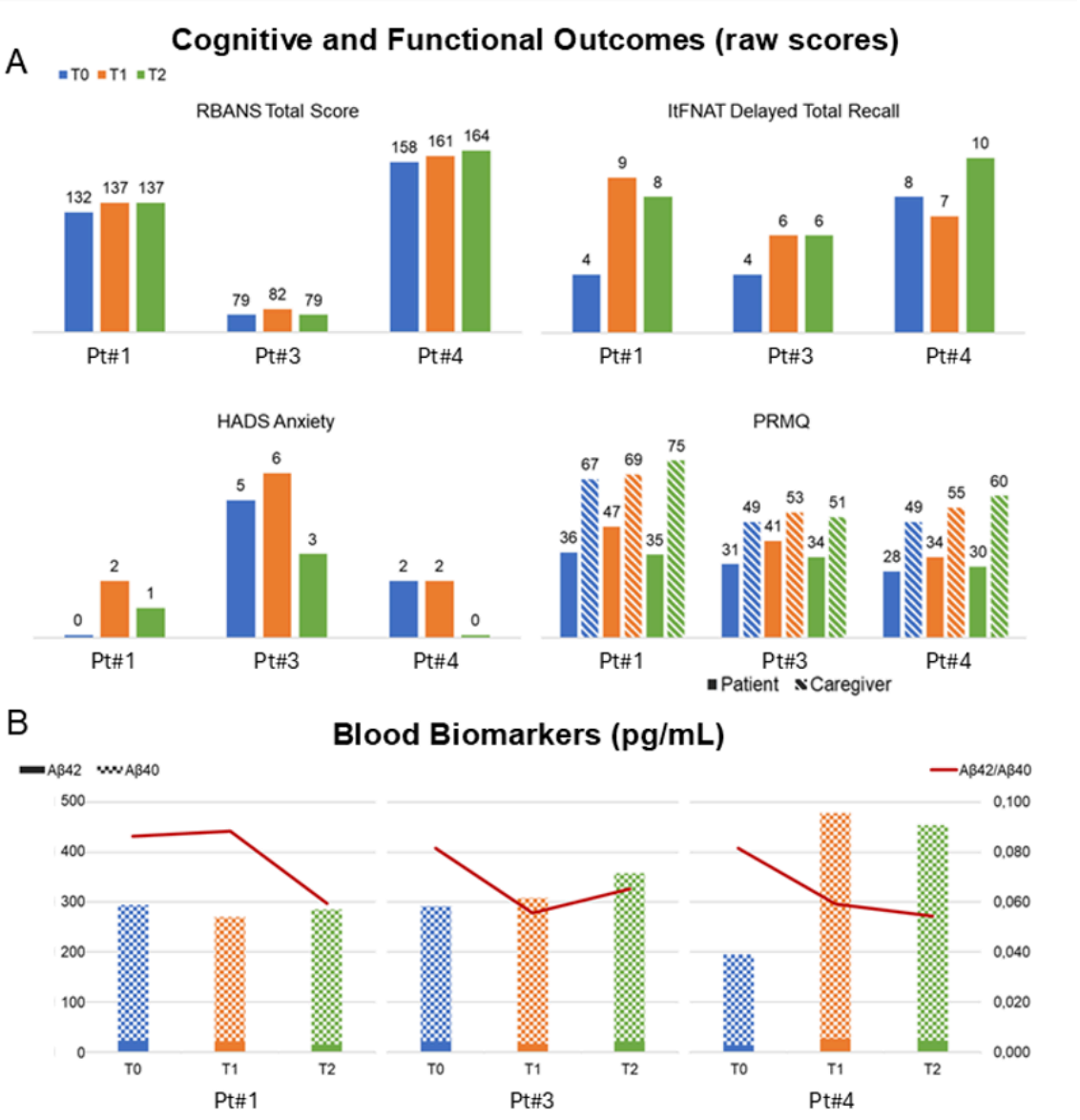


Figure 2

Cognitive, functional, and blood biomarker outcomes across time. A) Cognitive and Functional Outcomes of the three patients (Pt#1, Pt#3, Pt#4) assessed at baseline (T0, blue), immediately after the gamma-tACS intervention (T1, orange), and at the 3-month follow-up (T2, green). Bar plots display raw scores for RBANS Total Score, ItFNAT Delayed Total Recall, and HADS Anxiety. PRMQ scores are shown separately for patients (solid bars) and caregivers (hatched bars). Exact raw scores are reported above each bar. B) Blood biomarkers (pg/mL) across the same time points for each patient. Bars represent plasma Aβ42 (solid pattern) and Aβ40 (checkered pattern), plotted on the left y-axis, while the red solid line represents the Aβ42/Aβ40 ratio, plotted on the right y-axis. Changes across T0 (blue), T1 (orange), and T2 (green) are shown separately for each patient. RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; ItFNAT = Italian Face-Name Associative Test; HADS = Hospital Anxiety and Depression Scale; PRMQ = Prospective and Retrospective Memory Questionnaire.

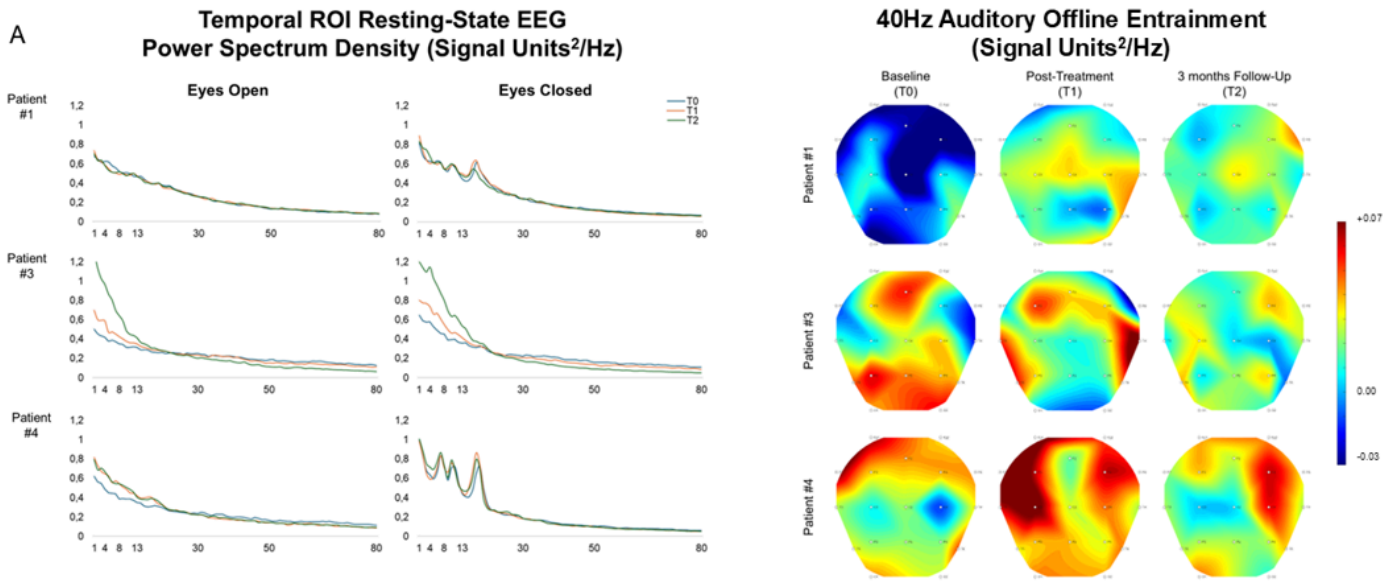


Figure 3

Electroencephalography outcomes. A) Temporal ROI resting-state EEG power spectrum density for eyes-open and eyes-closed conditions (in signal units^2/Hz) and B) Scalp plots showing offline entrainment following 5 minutes of 40 Hz auditory stimulation in the 38–42 Hz band for each patient at baseline (T0), after the gamma-tACS intervention (T1), and at the 3-month follow-up (T2).

Supplementary Files

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- Supplementarymaterials.docx