Exposure to gamma tACS in Alzheimer's disease: A randomized, double-blind, sham-controlled, crossover, pilot study

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ABSTRACT
Objective: To assess whether exposure to non-invasive brain stimulation with transcranial alternating current stimulation at γ frequency (γ-tACS) applied over Pz (an area overlying the medial parietal cortex and the precuneus) can improve memory and modulate cholinergic transmission in mild cognitive impairment due to Alzheimer's disease (MCI-AD).
Methods: In this randomized, double-blind, sham controlled, crossover pilot study, participants were assigned to a single 60 min treatment with exposure to γ-tACS over Pz or sham tACS. Each subject underwent a clinical evaluation including assessment of episodic memory pre- and post-γ-tACS or sham stimulation. Indirect measures of cholinergic transmission evaluated using transcranial magnetic stimulation (TMS) pre- and post-γ-tACS or sham tACS were evaluated.
Results: Twenty MCI-AD participants completed the study. No tACS-related side effects were observed, and the intervention was well tolerated in all participants. We observed a significant improvement at the Rey auditory verbal learning (RAVL) test total recall (5.7 [95% CI, 4.0 to 7.4], p < 0.001) and long delayed recall scores (1.3 [95% CI, 0.4 to 2.1], p = 0.007) after γ-tACS but not after sham tACS. Face-name associations scores improved during γ-tACS (4.3 [95% CI, 2.8 to 5.8], p < 0.001) but not after sham tACS. Short latency afferent inhibition, an indirect measure of cholinergic transmission evaluated with TMS, increased only after γ-tACS (0.31 [95% CI, 0.24 to 0.38], p < 0.001) but not after sham tACS.
Conclusions: exposure to γ-tACS over Pz showed a significant improvement of memory performances, along with restoration of intracortical connectivity measures of cholinergic neurotransmission, compared to sham tACS.

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Introduction
Alzheimer’s disease (AD) accounts for the vast majority of age-related dementias and it is estimated that approximately 100 million people worldwide will have AD dementia in 2050 [1]. Currently, there are no approved disease-modifying therapies for AD and treatments to slow the progression are urgently needed, particularly in the earliest disease stages when only episodic memory disturbances are present, namely in the mild cognitive impairment (MCI) phase.

AD is characterized by a prominent disruption of oscillations in the γ frequency band (30–80 Hz), which is proportional to the progression of AD [2,3] and is thought to be the result of the loss of...
inhibitory interneurons and cholinergic innervations, mediated by the accumulation of extracellular aggregates of toxic amyloid-beta peptides and tau-associated pathology [4–6]. An important set of recent studies have shown that the restoration of γ oscillations by neural entrainment in animal models of AD, induce a remarkable decrease in the pathological burden of amyloid and tau via increased microglial activity, resulting in a significant improvement of cognitive performances [7–9].

In humans, commonly applied external modalities to entrain brain activity include visual, auditory and somatosensory stimulation [10]. However, such approaches might offer limited impact in patients with AD given age-related impairment of sensory processing in elderly subjects (e.g., visual and hearing impairment) and the fact that sensory modulation is likely to primarily affect activity in specific brain networks that differ from those primarily affected by neurodegenerative pathology. Therefore, we chose to employ transcranial alternating current stimulation (tACS), which has been shown to allow entrainment of brain rhythms at specific frequencies in pre-defined cerebral regions, and thus modulate higher cognitive processes in healthy subjects [11–14].

We hypothesized that exposure to tACS delivered at γ frequency (γ-tACS) over Pz, an area overlaying the medial parietal cortex and the precuneus, a node of the default mode network [15] which is heavily affected even in the earliest phases of disease and may be easily targeted by transcranial stimulation, would be safe, well-tolerated, and induce significant improvement in memory in subjects with MCI due to AD [16,18].

To begin to test this hypothesis we carried out a pilot, double-blind, randomized, sham-controlled, crossover clinical trial, to assess the effects of exposure to γ-tACS on episodic memory functions. To gain some mechanistic insights, we also measured the effects of γ-tACS on short-latency afferent inhibition (SAI) by transcranial magnetic stimulation (TMS), a paradigm that indirectly assesses the function of cholinergic circuits [19].

Methods

Standard protocol approvals, registrations, and patient consents

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, #NP3847). The trial was registered at ClinicalTrials.gov (NCT04515433).

Participants

Participants fulfilling current criteria for MCI-AD [20] were recruited at the Center for Neurodegenerative Disorders, University of Brescia, Italy. For each subject, a review of past medical history, a semi-structured neurological examination, a standardized neuropsychological assessment and a structural imaging study were carried out.

MCI-AD diagnosis was corroborated by either cerebrospinal fluid (CSF) analysis supporting an AD pathological process (Aβ42 < 600 ng/L and tau > 400 ng/L) [21] or positive amyloid-PET scan [22].

At enrollment, the standardized neuropsychological assessment included the mini–mental state examination (MMSE) [23], short story [24], Rey complex figure (copy and recall) [25], letter and semantic fluencies [26], clock-drawing [27], and trail making test A/B [28,29]. Instrumental and basic activities of daily living (IADL/ BADL) were considered [30,31] and behavioral disturbances were evaluated with the neuropsychiatric inventory (NPI) [32].

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

Participants who were already on a pharmacologic regimen were allowed to receive treatment provided that the regimen remained unchanged for six weeks prior to the intervention, but initiation of drugs after the start of the observation period was not allowed (4 participants were on donepezil 10 mg QD, 2 participants were on zolpidem 10 mg QD, 1 patient was on trazodone 25 mg QD, 1 patient was on lorazepam 2.5 mg QD).

Study design

Participants were randomized into two groups in a 1:1 ratio, and each group received a single session of exposure to γ-tACS over Pz or a single session sham-tACS first and, after 1 week, stimulation was inverted (crossover phase) (see Fig. 1). Each TACS stimulation lasted 60 min.

The participants and the examiners performing clinical ratings, tACS 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. A computer-assisted block randomization was used to randomize subjects into groups that resulted in equal sample sizes.

In each session, a set of tasks tapping episodic memory performances was tested twice, at baseline (pre-stimulation) and after TACS (post-stimulation). Moreover, a memory task tapping associative memory performances was carried out during the last 20 min of TACS stimulation (see neuropsychological assessment below).

In each session, a 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 transcranial magnetic stimulation below).

The whole session, including cognitive and TMS testing before and after stimulation lasted approximately 150–180 min.

According to literature data, the effects of a single session of TACS are expected to last for 30–70 min [34]. Hence, participants were expected to return back to their initial clinical status between the two sessions of stimulation.

Experimental episodic memory assessment

To assess the effect of tACS on episodic memory performances, at each time-point (pre-stimulation and post-stimulation) of both exposure to γ-tACS and sham-tACS, the Rey auditory verbal learning (RAVL) (Rey A, 1964) test was carried out. RAVL test evaluates verbal episodic memory and is designed as a list-learning paradigm in which the subject hears a list of 15 nouns. The subject is asked to recall as many words from the list as possible after each of 5 repetitions of free-recall (total recall), and 20 min after an interference trial (long delayed recall) (Rey A, 1964). Different lists were randomized and used during pre- and post-stimulation to avoid learning effects.

To assess the effect of tACS on episodic associative memory, an unfamiliar face–name associations task (FNAT) [35] composed of an encoding and a retrieval phase was assessed during the last 20 min of TACS. During the encoding phase, a grey-scale picture of a face on a monitor with a proper name was presented, and the participant was required to respond if a male or female face was presented. During the retrieval phase, after 5 min from the encoding, the participant was shown a face with four proper names (i.e., the correct name and three other names), and the subject had
to associate the correct name to the face, as was presented during the encoding phase, as quickly as possible [36]. With respect to names, unfamiliar proper names were generated and randomly assigned to the unfamiliar faces. A set of 20 unfamiliar faces was presented at each tACS session, preceded by a training of 6 unfamiliar faces, with each presented in a random order. Gender of the stimuli were counterbalanced and randomized across blocks. Responses were collected via a response-box, and the stimuli remained on the screen until the response was made.

Transcranial magnetic stimulation assessment

A TMS figure-of-eight coil (each loop diameter 70 mm) connected to a monophasic Magstim BiStim® system (Magstim Company, Oxford, UK) was employed [37]. Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseus 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 point in which magnetic stimulation resulted in the maximum motor evoked potential (MEP) amplitude with the minimum stimulator intensity, as previously reported [38].

Short-latency afferent inhibition (SAI) was studied using a paired-pulse protocol, employing a conditioning-test design [39]. The test stimulus (TS) was adjusted to evoke a MEP of approximately 1 mV peak-to-peak amplitude, while the conditioning stimulus (CS) consisted of a single pulse (200 μs) of electrical stimulation at 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 implemented (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 s (±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. All of the participants were capable of following instructions and reaching complete muscle relaxation.

Exposure to gamma transcranial alternating current stimulation (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 x 6 cm). One electrode was placed with the center over Pz position according to the 10–20 international EEG coordinates (an area overlying the medial parietal cortex and the precuneus) and the other over the right deltoid muscle. This particular montage was chosen after performing computational modelling of electric field distribution hypothesizing that significant current densities would reach the precuneus, an area heavily affected even in the earliest phases of disease, and considering a previous study with partially similar montages, showing that tACS with an extracephalic electrode was the only one to significantly entrain brain oscillations (reported as phase stability) compared to other cephalic montages [40].

The electrodes were secured using elastic gauzes and electro-conductive 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/cm²) at a frequency of 40 Hz was applied for 60 min. For the sham condition, the electrode placement was the same, but the electric current was ramped down 60 s 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.

Computational modelling of electric field distribution

Based on methods developed previously by our group, we built a high-resolution (1 mm) MRI derived finite element model [41]. We considered a dataset from the ADNI project, rather than an arbitrary MRI as it better reflected the population considered in the study. 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

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**Fig. 1.** Study design. Legend: RAVL – Rey auditory verbal learning test; tACS – transcranial alternating current stimulation; TMS – transcranial magnetic stimulation.
equation was applied considering volume conduction. While current direction reverses at regular intervals, with Pz electrode serving as anode in one cycle and cathode in the other, the EF magnitude plot which indicates where the current is flowing does not change. We plotted cortical electric field (EF) magnitude on 3D-volume and on 2D-cross-sectional slices. The region corresponding to the precuneus was segmented and individually analyzed.

Outcome measures

The primary endpoints were defined as a) assessment of safety and tolerability, b) evaluation of blinding and c) the change from baseline in episodic and associative memory scores (RAVL total recall, RAVL long delayed recall and FNAT), compared to sham stimulation. The secondary endpoint was defined as changes from baseline in cholinergic transmission, evaluated indirectly with TMS.

Statistical analyses

We used a power analysis to determine the necessary sample size, based on previously published work on γ-tACS [42–47]. Considering that none of the cited studies adopted the same study design, that different populations were involved and the pilot nature of the study, we inferred from the cited studies an estimated partial η² of 0.12, equal to an effect size f of 0.369. Adopting a two-way repeated measures ANOVA, with 2 groups and 2 repeated measures each, with an α level of 0.05 and power (1-B) of 0.8, we identified a total sample size of 18. A final sample size of 20 was adopted to correct for possible dropouts.

Cohen’s Kappa was run to determine if there was 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 treatment on clinical scores and mean SAI over time we used a two-way or one-way repeated measure ANCOVA with TIME (baseline and post-treatment) and TREATMENT (sham vs γ stimulation) as within-subjects factors, and the order in which tACS was performed (real-sham or sham-real) as covariate. Post-hoc tests were performed after Bonferroni correction for multiple comparisons.

Spearman’s rank-order correlations were used to assess associations between the improvement in cognitive scores, neurophysiological parameters and clinical characteristics.

Statistical analyses were performed using SPSS version 21 (SPSS, Inc., Chicago, USA). Data are expressed as mean ± standard deviation, unless otherwise stated.

Data availability

All data, including outcome measure results, study protocol and statistical analysis plan, will be shared through ClinicalTrials.gov via public access (https://clinicaltrials.gov/ct2/show/NCT04515433).

Results

Twenty-four participants were initially screened, with three participants not meeting MCI criteria and one patient carrying an electronic implant (pacemaker). Twenty MCI-AD participants were enrolled and randomized to receive γ-tACS over Pz or sham stimulation first in a 1:1 ratio. All participants completed the study and were included in the final analysis (see Fig. 2). Demographic and clinical characteristics of included participants are reported in Table 1.

Computational modelling of electric field distribution

Computational modelling showed high current densities in the medial parietal cortex, in the precuneus, in the posterior cingulate cortex, in the occipital cortex, in the cerebellum and brainstem. After precuneus segmentation, which was the hypothesized target of γ-tACS, the maximum value of induced electric field was found to be 0.64 V/m, whereas the mean value of induced electric field was found to be 0.34 V/m (see Fig. 3).

Safety, tolerability and evaluation of blinding

No tACS-related side effects were observed, and tACS was well tolerated in 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 (κ = 0.00, p = 1.000). In particular, in the real stimulation group, 12 participants thought they were receiving real stimulation and 8 sham stimulation; in the sham stimulation group, 12 participants also thought they were receiving real stimulation and 8 sham stimulation. 6 participants thought they received real stimulation on both occasions (real and sham stimulation) while 2 thought they received sham stimulation both times.

Moreover, cutaneous sensations were equally perceived in both groups (z = −1.50, p = 0.132 at the Wilcoxon signed-rank test) and none of the participants reported phosphene/light flickering, suggesting that exposure to γ-tACS could not be distinguished from sham stimulation.

Episodic memory data: RAVL test and FNAT

Memory performances at baseline and at the end of the trials are reported in Table 2.

For RAVL test total recall scores, there was a statistically significant TIME × TREATMENT interaction at the repeated measures ANCOVA, (F (1,18) = 10.46, p = 0.005, partial η² = 0.37). Therefore, simple main effects were run. We observed a significant difference between real and sham tACS after stimulation (p < 0.001), with a mean difference of 5.7 (95% CI, 4.0–7.4), but not at baseline (p = 0.603). We observed a significant difference in the real tACS group after stimulation compared to baseline (p < 0.001), with a mean difference of 6.2 (95% CI, 3.7–8.6), but not in the sham tACS group (p = 0.912) (see Fig. 4, panel A).

For RAVL test long delayed recall scores, there was a statistically significant TIME × TREATMENT interaction at the repeated measures ANCOVA, (F (1,18) = 3.22, p = 0.023, partial η² = 0.16). Therefore, simple main effects were run. We observed a significant difference between real and sham tACS after stimulation (p = 0.007), with a mean difference of 1.3 (95% CI, 0.4–2.1), but not at baseline (p = 0.591). We observed a significant difference in the real tACS group after stimulation compared to baseline (p = 0.004), with a mean difference of 1.2 (95% CI, 0.4–1.9), but not in the sham tACS group (p = 0.847) (see Fig. 4, panel B).

For FNAT scores, there was a statistically significant difference between real and sham stimulation, (F (1,18) = 8.46, p = 0.009, partial η² = 0.32), with a mean difference of 4.3 (95% CI, 2.8–5.8) (see Fig. 4, panel C).

There was no significant association between baseline RAVL or FNAT scores and the extent of improvement in each test after tACS (all p > 0.005), suggesting that participants improved indistinctly of baseline impairment. The improvement at the RAVL test (delayed recall) after real tACS significantly positively correlated with the performance at the FNAT during real tACS (rₜ = 0.501, p = 0.024).

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Neurophysiological assessment

For SAI, there was a statistically significant TIME \times TREATMENT interaction at the repeated measures ANCOVA, ($F(1,18) = 9.50$, $p = 0.006$, partial $\eta^2 = 0.34$). Therefore, simple main effects were run. We observed a significant difference between real and sham tACS after stimulation ($p < 0.001$), with a mean difference of 0.31 (95% CI, 0.24–0.38), but not at baseline ($p = 0.969$). We observed a significant difference in the real tACS group after stimulation compared to baseline ($p < 0.001$), with a mean difference of 0.31 (95% CI, 0.26–0.37), but not in the sham tACS group ($p = 0.908$) (see Fig. 4, panel D).

There was no significant association between SAI at baseline and the percentage of improvement in RAVL test or FNAT (all $p < 0.05$), but there was a significant positive correlation between the increase in SAI after real stimulation and FNAT scores during real stimulation ($r_i = 0.611$, $p = 0.004$).

Discussion

In the present randomized, double-blind, placebo-controlled, crossover study, we observed that exposure to $\gamma$-tACS over Pz improves episodic memory performances and is associated with a restoration of SAI, an indirect marker of cholinergic transmission, in participants with MCI-AD, compared to sham stimulation. $\gamma$-tACS was well tolerated and did not induce any significant side-effects, as phosphenes, which seem to have a retinal origin and are perceived particularly with stimulation frequencies in the alpha-beta range [48–53].

MCI-AD is characterized by an impairment of episodic memory [54], which seems to be preceded by reduced power of oscillations in the gamma frequency band (30–120 Hz) [2,4,55]. Recent studies have suggested that manipulating neural oscillations may be effective in ameliorating AD pathology [7,56,57], and increasing gamma activity by neural entrainment in AD mouse models has shown a significant decrease in amyloid and tau pathology, associated with an increase in cognitive performances [7–9].

tACS is a device that applies a low-intensity sinusoidal electrical current at specific frequencies to the brain through electrodes on the scalp. Unlike tDCS, tACS does not alter neuronal excitability but directly interacts with ongoing neuronal activity leading to the entrainment or synchronization of brain network oscillations [58,59]. Studies have shown that distinct brain oscillations

Table 1

Demographic and clinical characteristics of MCI-AD subjects.

| Variable                      | MCI-AD       | Cut-off* |
|-------------------------------|--------------|---------|
| Age, years                    | 71.9 ± 7.0   | -       |
| Gender, female                | 50%          | -       |
| Duration of symptoms, years   | 2.8 ± 1.9    | -       |
| Education, years              | 8.9 ± 4.2    | -       |
| Family history for dementia   | 30%          | -       |
| Cognitive assessment          |              |         |
| MMSE                          | 25.6 ± 2.8   | >24     |
| Short Story                   | 6.3 ± 4.4    | >7.5    |
| Rey Complex figure, copy      | 23.5 ± 7.5   | >28.87  |
| Rey Complex figure, recall    | 5.3 ± 5.6    | >9.46   |
| Fluency, letter               | 26.3 ± 9.6   | >16     |
| Fluency, semantic             | 26.4 ± 10.1  | >24     |
| Trail Making test, part A (sec)| 73.2 ± 30.2  | <93    |
| Trail Making test, part B (sec)| 346.1 ± 153.7| <282   |
| Clock’s drawings              | 6.7 ± 3.1    | >5      |
| NPI                           | 5.4 ± 4.8    | -       |

MCI-AD = Mild Cognitive Impairment due to AD, MMSE = Mini-Mental State Examination; NPI = Neuropsychiatry Inventory.

Results are express as mean ± standard deviation, unless otherwise specified; *cut-off according to Italian normative data.
correlate to high-level cognitive functions including language processing, attention, working memory, and long-term memory formation [10]. In particular, gamma oscillations are prominent across multiple brain regions including the hippocampus, where they play a role in attentional selection and memory operations [60].

As brain oscillations are known to be associated with various brain functions, and gamma oscillations have been related to memory abilities, tACS may be able to enhance ongoing processes through exogenous augmentation and synchronization of cortical oscillations. Indeed, tACS has already been proven to be effective in increasing cognitive performances [61–63].

Improvements in declarative and associative memory were reported in studies delivering γ-tACS over the left dorsolateral prefrontal cortex [47,64] or after θ-tACS over temporoparietal regions [65–67], while β-tACS delivered over the left and right inferior frontal gyrus did not affect memory performance [17]. To our knowledge, only one published study has applied γ-tACS in participants with mild to moderate dementia, showing an improvement in memory tasks after stimulation of the left dorsolateral prefrontal cortex [69]. Another study has used high frequency

![Fig. 3. Computational modelling of electric field distribution. A) High-resolution MRI-derived finite element model with the exact experimental montage used in the study; B) 3-D volume sliced at the midline to reveal the medial surface and current flow pattern through the precuneus; C) cortical 2-D cross-section (sagittal) plots; D) topographical region corresponding to the precuneus that was segmented and individually analyzed; E) left precuneus; F) top view showing both left and right precuneus; G) right precuneus.]

### Table 2

Cognitive assessment scores and TMS measures before and after γ-tACS or sham stimulation.

| Variable | sham-tACS baseline | post-tACS | γ-tACS baseline | post-tACS |
|----------|--------------------|-----------|-----------------|-----------|
| Cognitive assessment | | | | |
| RAVL, total recall | 19.5 ± 6.6 | 19.4 ± 6.8 | 18.9 ± 8.4 | 25.1 ± 8.7<sup>a</sup> <sup>b</sup> |
| RAVL, long delayed recall | 1.5 ± 1.5 | 1.6 ± 1.5 | 1.7 ± 1.4 | 2.8 ± 1.9<sup>b</sup> |
| FNAT* | 5.4 ± 2.1 | 9.7 ± 2.1 | 9.7 ± 2.1 | 9.7 ± 2.1 |
| TMS | Mean SAI (0, +4 ms) | 0.85 ± 0.15 | 0.84 ± 0.16 | 0.85 ± 0.13 | 0.53 ± 0.09<sup>b</sup> |

Results are expressed as mean ± standard deviation. tACS = transcranial alternating current stimulation; RAVL = Rey auditory verbal learning test; FNAT = face–name associations task; SAI = short-latency afferent inhibition. *For FNAT, results are reported during stimulation.

<sup>a</sup> Significant difference compared to sham tACS.

<sup>b</sup> Significant difference compared to baseline; Bonferroni corrections for multiple comparisons have been applied after significant interaction at the one-way or two-way repeated measures ANCOVA.
repetitive TMS (rTMS) targeting the precuneus, showing a selective improvement in episodic memory, associated with a change of functional connections between the precuneus and medial frontal areas within the default mode network [70]. Taken together, these studies highlight how memory performances may be effectively modulated by non-invasive brain stimulation in both healthy adults and participants with mild to moderate dementia.

In the present study we evaluated both online and offline effects of γ-tACS with the FNAT and RAVL test, respectively. Typically, entrainment of cortical oscillations inducing neural synchronization is described as one of the main mechanisms of tACS, which inherently relates to online effects. However, more recently, spike-timing dependent plasticity has been proposed to explain offline tACS effects, with effects depending on the intrinsic natural frequency of recurrent networks in a particular brain region [12,71–75]. State-dependency probably has an important aspect also for tACS, as already shown in the field of TMS [76]. Thus, the effects observed for FNAT scores might be also influenced by an active engagement during stimulation and future studies should be designed to control for this aspect.

The improvement observed at the FNAT, which has been shown to depend primarily on hippocampal structures [77], could be explained by the entrainment of large-scale cortical network activity by network resonance, functionally connected to the precuneus via the default mode network [58].

The present study confirms and extends previous findings on the role of γ-tACS in restoring cognitive functions also in participants with MCI-AD. Compared to previous work, we a) employed a crossover design to avoid confounding factors and reduce error variance, b) we included strictly selected MCI-AD subjects, with a comprehensive clinical and biomarkers assessment; c) we used γ-tACS, which allows to non-invasively entrain neural oscillations as compared to other transcranial electrical stimulation techniques [78], d) we selected the medial parietal cortex, an area overlying the precuneus, as the target area to be stimulated, one of the first regions to be affected in prodromal AD and the hub of the default

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**Fig. 4.** A) RAVL total recall, B) RAVL long delayed recall, C) FNAT scores and D) SAI measures before and after exposure to γ-tACS or sham stimulation.

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. For SAI, red lines represent average reference values from a group of Alzheimer’s disease patients (n = 273) with standard deviations (red dashed lines), while blue lines represent average reference values from a group of healthy controls (n = 147) with standard deviations (blue dashed lines), obtained from Ref. [38]. Error bars represent standard errors. **significant difference compared to sham tACS; †significant difference compared to baseline; Bonferroni corrections for multiple comparisons have been applied after significant interaction at the one-way or two-way repeated measures ANCOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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mode network, functionally connected to mesial temporal regions, while being easily accessible by transcranial stimulation; e) we tested episodic but also associative memory, which is impaired early in the prodromal phase of the disease and plays a considerable role in the quality of everyday life, and f) we further supported clinical findings with indirect measures of cholinergic neurotransmission.

Taken together, these results provide novel evidence that exposure to non-invasive treatment with tACS over Pz represents an effective strategy to enhance memory in MCI-AD.

The clinical results herein presented were corroborated by intracranial connectivity parameters, as measured by TMS, with a clear restoration of SAI in participants after exposure to γ-tACS compared to participants who underwent sham stimulation. SAI is considered to rely on central cholinergic circuits, as it is decreased or eliminated by applying scopolamine, a muscarinic agonist, in healthy controls [79], and SAI has been demonstrated a reliable diagnostic marker of AD since the earliest disease stages [80–83]. The restoration of cholinergic circuits after exposure to γ-tACS may explain its effect on cognitive performances.

We acknowledge that the present pilot study entails some limitations: our group of participants was relatively small, even though well characterized, and larger samples are needed to corroborate the present findings; multiple sessions need to be considered in future studies to assess long-lasting effects; predictors of clinical response, such as Apolipoprotein E genotype or variables of cognitive reserve, need to be assessed; potential cognitive effects could be explained also by an off-target stimulation of other than the hypothesized target, the precuneus. Indeed, while the current flow maps indicate high electric field values in the occipital cortex, cerebellum, posterior cingulate cortex and brainstem, there is substantial electric field induced in the precuneus as well. It has been widely demonstrated across several modelling efforts that 2 mA of transcranial electrical stimulation results in electric field magnitude of 0.5–1 V/m in the cortex and is therefore considered “efficacious dose” [84,85]. Our modelling results (looking individually at the precuneus) confirm that a relevant dose is in fact induced in the structure. Any transcranial electrical stimulation is characterized by current flow through the entire path between the stimulation electrodes due to the physics of stimulation. As a result, it is expected that additional non-target structures will receive high electric field. Moreover, the montage chosen in this study, with an extracephalic return electrode, has been shown to significantly entrain cerebral oscillations compared to other cephalic montages in a similar study [40]. Considering that associative and episodic memory are deeply associated with the precuneus [86], the clinical effects observed after exposure to γ-tACS might also be explained by an off-target stimulation of structures other than the precuneus. These aspects highlight how tACS might also be explained by an off-target stimulation of the precuneus as well. It has been widely demonstrated across studies that the precuneus is involved in the mode network, functionally connected to mesial temporal regions, while being easily accessible by transcranial stimulation; e) we tested episodic but also associative memory, which is impaired early in the prodromal phase of the disease and plays a considerable role in the quality of everyday life, and f) we further supported clinical findings with indirect measures of cholinergic neurotransmission.

In conclusion, the results herein observed on clinical assessments and intracortical connectivity measures suggest that γ-tACS will undoubtedly be of interest in clinical practice in the near future, largely due to the excellent benefit-risk ratio. Research efforts in this direction should help develop future non-pharmacological interventions, which have the potential to be delivered by the participants themselves or their caregivers in at-home settings, aimed at reducing cognitive deficits in the prodromal phases of AD.

Author contributions

A.B. and B.B. contributed to the conception and design of the study. A.B., V.C., M.S.C., M.C., C.B., D.A., C.T., E.S., A.P.L., B.B. contributed to acquisition, analysis of data, and significant critical review of the manuscript. A.B., A.P.L., B.B. contributed drafting the text.

CRediT authorship contribution statement

Alberto Benussi: Conceptualization, Methodology, Formal analysis, Investigation, Writing — original draft, Visualization. Valentina Cantoni: Investigation, Writing — review & editing. Maria Sofia Cotelli: Investigation, Writing — review & editing. Maria Cotelli: Investigation, Writing — review & editing. Chiara Brattini: Investigation, Writing — review & editing. Abhishek Datta: Methodology, Investigation, Writing — original draft. Chris Thomas: Methodology, Investigation, Writing — review & editing. Emiliano Santarneccchi: Investigation, Writing — review & editing. Alvaro Pascual-Leone: Methodology, Investigation, Writing — original draft. Barbara Borroni: Conceptualization, Methodology, Formal analysis, Investigation, Writing — original draft, Visualization, Supervision, Project administration.

Declaration of competing interest

Alberto Benussi, MD was partially supported by the Airalzh-AGYR2020; and is listed as an inventor on issued and pending patents on the use of non-invasive brain stimulation for the differential diagnosis of dementia and to increase cognitive functions in patients with neurodegenerative disorders. Maria Sofia Cotelli, MD received financial support by the Italian Ministry of Health (Ricerca Corrente), Emiliano Santarneccchi, PsDy, PhD: is consultant for Neuroelectrics and Neurocare Group Italy. Alvaro Pascual-Leone, MD, PhD is a co-founder of Linus Health and TI Solutions AG; serves on the scientific advisory boards for Starlab Neuroscience, Neuro-electrics, Magstim Inc., Nexstim, Cognito, and MedRhythms; and is listed as an inventor on several issued and pending patents on the real-time integration of noninvasive brain stimulation with electroencephalography and magnetic resonance imaging. Dr. Pascual-Leone was partly supported by the National Institutes of Health (R01MH100186, R21AG051846, R01MH111875, R01MH115949, R01 MH117063, R24AG06142, and P01 AG031720), the National Science Foundation, DARPA, and the Barcelona Brain Health Initiative (La Caixa and Institute Guttmann). Barbara Borroni, MD, is listed as an inventor on several issued and pending patents on the use of non-invasive brain stimulation for the differential diagnosis of dementia and to increase cognitive functions in patients with neurodegenerative disorders. Valentina Cantoni, MS, Maria Cotelli, PhD, Chiara Brattini, MS, Abhishek Datta, PhD, Chris Thomas, BS, report no disclosures.

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