Alzheimer's
Research & Therapy

RESEARCH

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Effects of 52 weeks of precuneus rTMS in Alzheimer's disease patients: a randomized trial

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Abstract

Background Personalized repetitive transcranial magnetic stimulation (rTMS) of the precuneus (PC) is emerging as a new non-invasive therapeutic approach in treating Alzheimer's disease (AD).

Here we sought to investigate the effects of 52 weeks of rTMS applied over the PC on cognitive functions in patients with mild-to-moderate dementia due to AD.

Methods Forty-eight patients with mild-to-moderate dementia due to AD were enrolled for the study. Of those 31 patients were extended to 52 weeks after being included in a 24-week trial (NCT03778151) with the same experimental design. The trial included a 52-week treatment with a 2-week intensive course where rTMS (or sham) was applied over the PC daily (5 times per week, Monday to Friday), followed by a 50-week maintenance phase in which the same stimulation was applied once weekly. Personalization of rTMS treatment was established using neuronavigated TMS in combination with electroencephalography (TMS-EEG). The primary outcome measure was change from baseline to week 52 of the Clinical Dementia Rating Scale–Sum of Boxes (CDR-SB). Secondary outcomes included score changes in the Alzheimer's Disease Assessment Scale– Cognitive Subscale $(ADAS\text{-}Cog)_{11}$, Mini Mental State Examination (MMSE), Alzheimer's Disease Cooperative Study–Activities of Daily Living scale (ADCS-ADL) and Neuropsychiatric Inventory (NPI). Changes in cortical activity and connectivity were monitored by TMS-EEG.

Results Among 48 patients randomized (mean age 72.8 years; 56% women), 32 (68%) completed the study. Repetitive TMS of the PC (PC-rTMS) had a significant effect on the primary outcome measure. The estimated mean change in CDR-SB after 52 week was 1.36 for PC-rTMS (95% confidence interval (CI) [0.68, 2.04]) and 2.45 for sham-rTMS group (95%CI [1.85, 3.05]). There were also significant effects for the secondary outcomes $ADAS\text{-}Cog_{11}$, ADCS-ADL and NPI scores. Stronger DMN connectivity at baseline was associated with favorable response to rTMS treatment.

Conclusions Fifty-two weeks of PC-rTMS may slow down the impairment of cognitive functions, activities of daily living and behavioral disturbances in patients with mild-to-moderate AD. Further multicenter studies are needed to confirm the clinical potential of DMN personalized rTMS.

Trial registration The study was registered on the clinicaltrial.gov website on 07–07-2022 (NCT05454540).

Keywords Alzheimer's disease, Precuneus, rTMS, Default mode network

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Background

Noninvasive brain stimulation (NIBS) methods, such as personalized repetitive Transcranial Magnetic Stimulation (rTMS), are emerging as novel therapeutic strategies to counteract cognitive dysfunction in patients with Alzheimer's Disease (AD) [1]. In particular, the Precuneus (PC) has been recently identified as the ideal rTMS target for stimulation to slow down cognitive and functional decline in AD [2, 3]. The PC is a key node of the Default Mode Network (DMN) and it is the earliest region to be affected by amyloid deposition [4, 5] as well as by gray matter loss, and functional connectivity disconnection between regions and organizations within networks [6, 7]. rTMS enhances mechanisms of long-term plasticity that are altered in AD patients since the early stages of the disease [8, 9]. Moreover, in animal models of AD, rTMS decreases Aβ and phosphorylated tau deposits, increases neurogenic proteins such as brain-derived neurotrophic factor, and reduces pro-inflammatory cytokines such as IL-6 and TNF-α [10–12]. We recently performed a phase-2 trial showing that 24 weeks of personalized PC-rTMS slow down decline of cognitive functions and functional activities of daily living as compared to sham in patients with mild-to-moderate AD.

Here, we sought to determine whether extended treatment up to 52 weeks may still result in preserved cognition and function over a longer period, up to one year. Hence, we performed a pilot trial to evaluate safety and efficacy of PC-rTMS in mild-to-moderate AD patients when applied over a period of 52 weeks.

Methods

Clinical study design

This was a monocentric, sham-controlled, randomized, and double-blind pilot trial of PC-rTMS in patients with mild-to-moderate dementia due to AD. The study was conducted in a research hospital in Italy (Santa Lucia Foundation IRCCS). The trial was approved by the review board and the local ethics committee of the Santa Lucia Foundation IRCCS (Prot. CE/Prog. 716) in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. All patients or their relatives or legal representatives provided written informed consent. Patients could withdraw at any point without prejudice. This report followed the CONSORT reporting guideline for randomized studies. The study was registered on the clinicaltrial.gov website on 07–07-2022 (NCT05454540). An independent committee monitored the patients' safety according to the Data Monitoring Committee Charter.

Patients were eligible if they had an established diagnosis of probable mild-to-moderate AD according to the International Working Group recommendations [13]. AD patients aged $>50 \leq 85$ years; had a Clinical Dementia Rating (CDR) [14] score of 0.5–1; a Mini Mental State Examination (MMSE) [15] score of 18–26 at screening; cerebrospinal fluid biomarker evidence of amyloid and tau pathology [13] or PET positive for amyloid; had one caregiver; had been treated with acetylcholinesterase inhibitor for at least 6 months. Patients were excluded if they had extrapyramidal signs, history of stroke, other neurodegenerative disorders, psychotic disorders and if they had been treated six months before enrollment with antipsychotics, antiparkinsonian, anticholinergics and antiepileptic drugs.

Randomization and masking

A total of 48 randomly assigned patients were planned on the basis of our previous study in which we assessed the effects of PC rTMS on cognitive functions in a small sample of prodromal AD patients [2]. In that pilot study, a significant difference was observed in pre-post PC rTMS treatment (of 2 weeks) in $n=14$ patients for the Rey Auditory Verbal Learning Task (RAVLT) (mean pre = 2.2, SD: 2.7; mean post = 3.0, SD = 2.6; corresponding to an effect size of about 0.39 for a Wilcoxon signed-rank test –matched pairs-, with hypothesized correlation pre-post of rho = 0.7). Treatment duration of the present study is more than ten times larger than the one of the pilot studies thus it is plausible to expect an effect size at least twice larger of the one found in the pilot study, i.e. of about 0.75. With this effect size, adopting a two-tailed paired Wilcoxon signed-rank, with type I error alpha = 0.05 and a plausible correlation between pre-post measured variables of 0.7, the minimum sample for reaching a power of 0.8 is estimated equal to $n=34$; and up to $n=46$ to ensure a power of 0.9. Precautionarily, the minimum total sample size was set at $n=48$ to ensure adequate size for within groups analysis as well. Randomization was performed and assigned independently by an external statistician, held centrally, and not divulged to any other person involved in the trial. Study groups were balanced in terms of age, sex, education and APOE carriers with a covariate-adaptive randomization procedure taking into account patients that were extended from the previous 24 weeks trial or newly enrolled patients. AD patients were enrolled by expert neurologists (GK, MA, FDL, CM) who were blinded to treatment allocation. Clinical evaluations were performed by expert neurologists and neuropsychologists (SB, IB, MA), who were blinded to treatment allocation, at the beginning of the treatment (W0) and after 12 (W12), 24 (W24), 36 (W36) and 52 weeks (W52). rTMS sessions were performed by dedicated technicians (MF, AD). Neurophysiological evaluations were performed by means of TMS-EEG and

EEG by expert neurophysiologists (EPC, MM, RE, VP), blinded to treatment allocation, at the beginning of the treatment (W0) and after 52 weeks (W52). At each clinical visit (or upon early termination), adverse events (AEs) were recorded, vital signs measured, and physical and neurological examination were performed.

Trial procedures

The pilot trial included a 52-week treatment, with a 2-week intensive course where PC-rTMS (or sham-rTMS) was applied over the PC daily (5 times per week, Monday to Friday), followed by a 50-week maintenance phase in which the same stimulation was applied weekly. rTMS was carried out using a Magstim Rapid2 magnetic biphasic stimulator connected with a 70-mm diameter figure-of-eight coil (Magstim Company, Whitland, UK). Each rTMS session consisted of forty 2-s trains delivered at 20 Hz that were spaced-out by 28 s (number of stimuli for each session: 1600). This protocol lasted approximately for 20 min [2]. Throughout the entire 52-week period, a total of 96.000 stimuli were delivered for each patient across 60 sessions. The TMS coil position was constantly monitored using a neuronavigation system coupled with an infrared camera. rTMS sham treatment was applied with a sham coil positioned in correspondence to the target area. Coil was orientated parallel to the midline to induce a posterior-anterior (PA) directed current. Personalization of rTMS treatment was established using single-pulse TMS in combination with a 64-channel electroencephalography (TMS-EEG) based on the evaluation of TMS-evoked potentials (TEPs) [2] to determine the intensity and location of stimulation. Each patient preliminarily underwent a series of TMS-EEG recordings over a site corresponding to the PC, identified based on previous fMRI works [16, 17]. Intensity of stimulation was initially set at 100% of the scalp-to-cortex adjusted resting motor threshold and adjusted until the first TEP component showed a peak-to-peak amplitude of at least 4–6 μV [3, 18]. Target location optimization was further achieved by identifying the scalp location generating the highest response to TMS for each patient via a grid search over a 3×3 cm area centered around the original fMRI-defined stimulation target (MNI coordinates: x=0, y=-65, z=45) [16, 17] (see Supplementary methods).

Outcomes measures

The primary outcome measure was the change at 52 weeks (W52) from baseline (W0) of the Clinical Dementia Rating Scale Sum of Boxes (CDR-SB) score (CDR-SB scores range from 0 to 18, with higher scores indicating worse cognition and daily function) [19]. The intention-to-treat analysis set included all patients who were randomized in the study.

The secondary outcome measures included the change at 52 weeks (W52) from baseline (W0) of the following tests: 1) Alzheimer's Disease Assessment Scale – Cognitive Subscale (ADAS-Cog)11 [20]; 2) MMSE score [21]; 3) Activities of Daily Living (ADCS-ADL) [22]; 4) Frontal Assessment Battery (FAB) [23]; 5) Neuropsychiatric Inventory (NPI) [24]. We also considered, as exploratory outcomes, neurophysiological measures from TMS-EEG and EEG evaluations (technical details on EEG and TMS-EEG evaluations are reported in the supplementary methods). TMS-EEG was used to assess cortical excitability, by means of TEPs [25], and DMN effective connectivity, by computing the TMS-evoked EEG activity in source space from the stimulated precuneus to the medial-frontal cortex [26]. Resting EEG was used to assess the oscillatory activity throughout the scalp.

Statistical analysis

Statistical analysis was run with R version 3.6.1. Normal distribution of end-point variables was assessed by means of Shapiro-Wilks' test. The level of statistical significance was set at $\alpha = 0.05$. Homogeneity between the means in the baseline characteristics between the two groups were assessed with independent t-test, Mann–Whitney test or χ^2 depending on the type of variable (categorical or continuous) and its distribution.

The longitudinal assessment of the end points across groups was performed through generalized linear mixed models (GLMM), depending on data distribution, for repeated measures, with a random intercept varying for each patient to account for individual differences at baseline and for changes at follow-up points. The dependent variables for the models were: CDR-SOB; $ADAS\text{-}COG_{11}$, MMSE, ADCS-ADL, NPI and FAB. The independent fixed factors were "rTMS" (real vs. sham), "time" (W0; W12; W24; W36; W52) and their interaction. To test for possible effects of age and education we inserted these variables as covariates in all the models. Thus, we conducted a total of six GLMM, separately for each clinical test, with general equation:

$$Clinical\ test \sim 1 + rTMS + time + age + education + rTMS : time + (1|patient) \quad (1)$$

Significant effects of GLMM analyses were further evaluated with simple contrasts and a simple effect moderator analysis. Simple contrast analysis compared the dependent variable values at the baseline level (W0)

with all the subsequent follow-ups; this analysis was conducted to observe if rTMS treatments produced significant changes in variables across the full-time course. Simple effect moderator analysis was conducted to investigate the presence of a time effect, i.e. a significant change in the clinical score among the follow-ups, moderate by the group, i.e. real-rTMS or sham-rTMS.

Analysis of TMS-EEG and EEG outcomes was performed with the same GLMM approach, using the following dependent variables: TEP peak-to-peak amplitude; TMS-EEG source activity propagation and EEG spectral power in the frequency bands (theta (4–7 Hz), alpha (7–13 Hz), beta (13–30 Hz) and gamma (30–50 Hz)). The independent fixed factors were "rTMS" (real vs. sham), "time" (W0; W52) and their interaction. Finally, we computed bivariate linear correlations to examine possible linear relations between the primary clinical outcome change and neurophysiological changes.

Results

Forty-eight patients underwent randomization between February 1, 2018, and April 30, 2022. Of those 31 patients accepted to extend treatment duration up to 52 weeks after being included in a 24-week trial (NCT03778151) with the same experimental design [3]. Patients that agreed to participate in the extension phase were kept on the same arm of treatment (PC-rTMS vs sham-rTMS). 24 additional AD patients that did not participate previously to other rTMS study were screened of which 17 underwent randomization and were allocated either to rTMS or sham using an adaptive randomization design (Fig. 1). In total 27 patients were allocated to PC-rTMS and 21 to sham rTMS. Table 1 reports the demographic characteristics of the two groups and their clinical scores at the first visit (W0). The mean age of the total sample of patients was 72.8 years (SD = 5.26, range 62 to 88), of which 56% were female. Patients had a mean MMSE raw score at baseline of 21.3 (SD = 2.96). The baseline patients' demographics and clinical characteristics did not differ between the PC-rTMS and sham-rTMS groups. Mean rTMS treatment intensity (% maximal stimulator output-MSO) was 55.92 (SD = 11.53) in the PC-rTMS group and 53.23 (SD = 6.31) in the sham-rTMS group.

Safety

The procedure was safe with mild adverse events resolved on the day of occurrence with either minor or no action. The incidence of adverse events was similar across dose groups (Table 2). The incidence and type of adverse

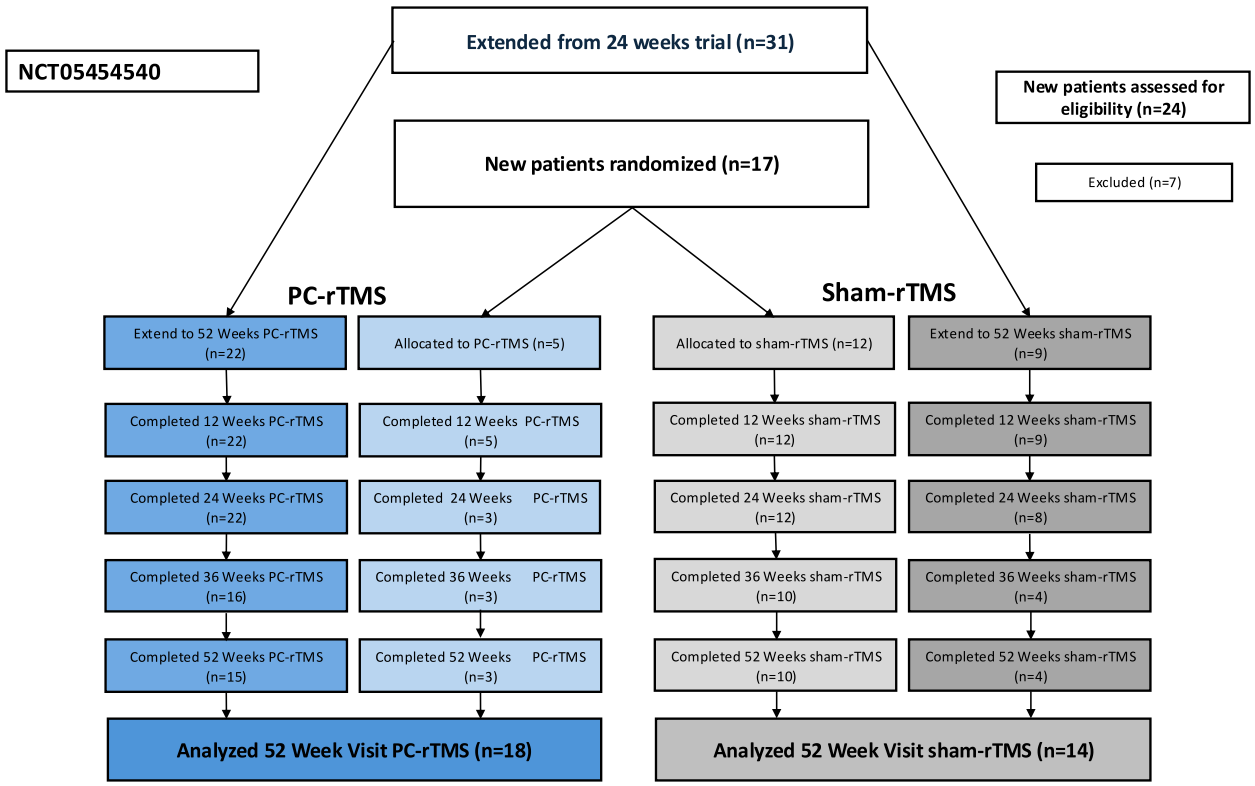

Fig. 1 Flow diagram of the trial. Randomization, trial-group assignment, and follow-up in the trial

Table 1 Baseline patients' demographics and clinical characteristics

	PC-rTMS (*N* = 27)	Sham-rTMS (*N* = 21)	Group differences (*p*-value)
Age, mean (SD)	74 (5.11)	71.2 (5.11)	*p* = 0.062
Sex, Female, No (%)	16 (59.2%)	11 (52.3%)	*p* = 0.634
Education, years, mean (SD)	10 (4.78)	11.5 (3.6)	*p* = 0.241
rTMS intensity, maximum stimulator output (%)	**55.9 (11.53)**	**53.2 (6.31)**	***p* = 0.566**
MMSE raw score, mean (SD)	20.9 (2.87)	21.9 (3.07)	*p* = 0.285
CDR-SB raw score, mean (SD)	3.69 (1.48)	3.86 (1.31)	*p* = 0.677
ADAS-Cog raw score, mean (SD)	22.8 (6.86)	23.3 (7.05)	*p* = 0.788
ADCS-ADL score, mean (SD)	59.3 (9.28)	59.4 (9.47)	*p* = 0.972
NPI score, mean (SD)	9.07 (10.1)	15.2 (8.36)	*p* = 0.03
FAB raw score, mean (SD)	11.3 (3.53)	10.3 (3.12)	*p* = 0.306

Table 2 Adverse events reported

Adverse Events	rTMS (number of patients/%)	Sham (number of patients/%)
Mild headache	3 (11.5%)	1 (4.5%)
Skin discomfort	2 (7.7%)	2 (9%)
Scalp discomfort	4 (15.4%)	2 (9%)
Neck pain	1 (3.8%)	2 (9%)
Stiffness	0 (0%)	1 (4.5%)

events were consistent with those expected in rTMS clinical studies in AD patients. Due to COVID pandemics a total of 15 patients (8 in the PC-rTMS group and 7 in the sham-rTMS group) discontinued the study. One patient in the PC-rTMS group discontinued because of lack of caregiver support.

Primary outcome measure

The mean baseline CDR-SB total score was 3.74 (SD = 1.47) for the PC-rTMS group, and 3.90 (SD = 1.33) for the sham-rTMS group, there was no difference between the two groups [t(46) = -0.418; $p = 0.67$] (Table 1). Figure 2 depicts the GLMM estimated mean changes at all the time points. Table 3 report the details of the target endpoint at 52 weeks. GLMM on CDR-SB scores showed a significant result in terms of time main effect ($p < 0.001$) and time × group interaction ($p = 0.038$). Patients receiving PC-rTMS generally showed a slower decline compared to the sham-rTMS group at W36 ($p = 0.041$) and W52 ($p = 0.007$). The estimated mean change at W52 was 1.36 for PC-rTMS group (95% confidence interval (CI) [0.680, 2.039]) and 2.45 for sham-rTMS group (95% CI [1.847, 3.052]).

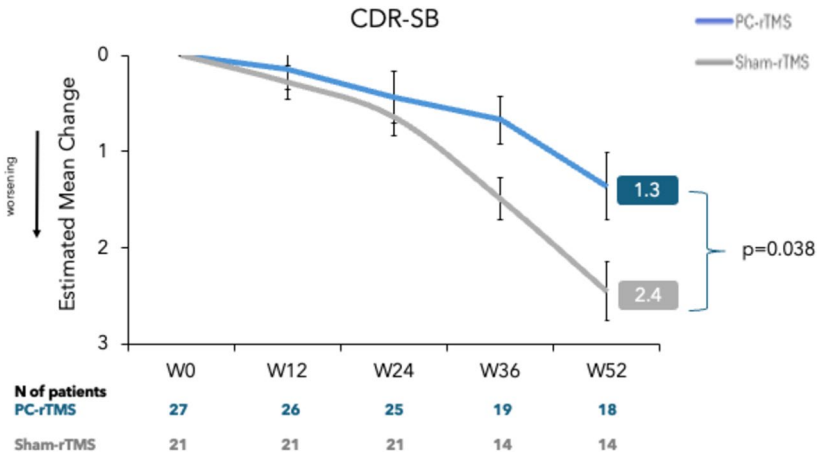

Fig. 2 Primary outcome measure. Estimated mean group changes for clinical scores. Estimated mean group changes from baseline (W0) in the CDR-SB following 12 weeks (W12), 24 weeks (W24) 36 weeks (W36) and 52 weeks (W52) of PC-rTMS and sham-rTMS. Y-axis of each outcome was adapted in order to considering all depicted descending trend as a worsening. In the CDR-SB scale; scores are obtained by summing each of the domain box scores, with scores ranging from 0 to 18, with higher scores indicating worse cognition. Baseline is plotted at Week 0, which is the baseline measurement before the first rTMS session. Error bars indicate standard errors

Table 3 GLMM estimated effects of changes in Primary and Secondary Outcomes from Baseline to Week 52

Outcome	PC-rTMS Mean [95%CI]	Sham-rTMS Mean [95%CI]	rTMS*group interaction F-test	rTMS*group interaction *p*-value
CDR-SB	1.36 [0.68 2.04]	2.45 [1.85 3.05]	$F_{4,144.9}=2.60$	**0.038***
ADAS-Cog [a]	5.9 [3.72 8.08]	10.4 [7.34 13.46]	$F_{4,139.7}=2.95$	**0.022***
MMSE [a]	-1.1 [-2.43 0.23]	-3.9 [-5.87 -1.93]	$F_{4,141.9}=2.43$	0.051
ADCS-ADL	-1.5 [-4.51 1.51]	-11.6 [-14.97 -8.23]	$F_{4,140.4}=6.70$	**<0.001***
NPI	3.28 [-0.42 6.98]	6.91 [3.11 10.71]	$F_{4,147.6}=2.44$	**0.049***
FAB [a]	-0.89 [-2.28 0.5]	-0.85 [-2.76 1.06]	$F_{4,140.8}=1.59$	0.180

[a] GLMM adjusted for age and education

* indicates statistically significant values ($p<0.05$)

Secondary outcomes measures

The mean baseline scores and the relative standard deviation of the secondary outcomes are reported in Table 1, we did not observe any difference between the two groups (all ps > 0.05). Table 3 reports the details at the main endpoint at 52 weeks.

GLMM on ADCS-ADL scores showed a significant result in terms of time main effect ($p<0.001$), group main effect ($p=0.035$) and a significant time × group interaction ($p<0.001$). Patients in the PC-rTMS group showed a stable performance while the sham-rTMS group showed a decline from the W24 follow-up (W12-W0: $p=0.054$; W24-W0: $p=0.006$; W36-W0: $p<0.001$; W52-W0: $p<0.001$). When comparing the two groups, patients receiving PC-rTMS showed different scores compared to the sham-rTMS group at all the time points with higher ADCS-ADL scores (W12: $p=0.010$; W24: $p=0.013$; W36: $p=0.003$; W52: $p<0.001$). The estimated mean change for W52 evaluation was -1.5 for PC-rTMS group (95% confidence interval (CI) [-4.510, 1.511]) and -11.6 for sham-rTMS group (95% CI [-14.971, 8.232]) (Fig. 3A).

GLMM on ADAS-Cog scores showed a significant result in terms of time main effect ($p<0.001$) and a significant time × group interaction ($p=0.022$). Patients receiving PC-rTMS showed a slower decline compared to the sham-rTMS group at all the time points with higher ADAS-Cog score (W12: $p=0.026$; W24: $p=0.014$; W36: $p=0.006$; W52: $p=0.007$). The estimated mean change at W52 was 5.9 for PC-rTMS group (95% confidence interval (CI) [3.718, 8.081]) and 10.4 for sham-rTMS group (95% CI [7.336, 13.463]) (Fig. 3B).

GLMM on MMSE scores showed a significant result in terms of time main effect ($p<0.001$) and a trend for a significant time × group interaction ($p=0.051$). Patients in the PC-rTMS group showed a stable performance, compared to the W0 evaluation, with no effect of time in their MMSE scores ($p=0.296$), whereas this was not true for the sham-rTMS group, which showed a progressive decline (W12-W0: $p=0.451$; W24-W0: $p=0.043$; W36-W0: $p=0.006$; W52-W0: $p<0.001$). The estimated mean change for W52 evaluation was -1.1 for PC-rTMS group (95% confidence interval (CI) [-2.428, 0.228]) and -3.9 for sham-rTMS group (95% CI [-5.868, -1.931]) (Fig. 3C).

GLMM on NPI scores showed a significant result in terms of time main effect ($p<0.001$) and a significant time × group interaction ($p=0.049$). Patients in the PC-rTMS group showed a stable performance, compared to the W0 evaluation, with no effect of time in their NPI scores ($p=0.157$) whereas the sham-rTMS group showed a progressive decline (W12-W0: $p=0.009$; W24-W0: $p<0.001$; W36-W0: $p<0.001$; W52-W0: $p<0.001$). When comparing the two groups, patients receiving PC-rTMS showed a slower decline compared to the sham-rTMS group at W12 ($p=0.024$); W24 ($p=0.009$); W36 ($p=0.014$) but not at W52 ($p=0.122$), with higher NPI score. The estimated mean change for W52 evaluation was 3.28 for PC-rTMS group (95% confidence interval (CI) [-0.422, 6.982]) and 6.91 for sham-rTMS group (95% CI [3.114, 10.705]) (Fig. 3D). To further explore the rTMS effects at NPI subitem-level, we performed independent t-test between the W52-W0 score differences of the two groups for each NPI subitem. Among the NPI subitems,

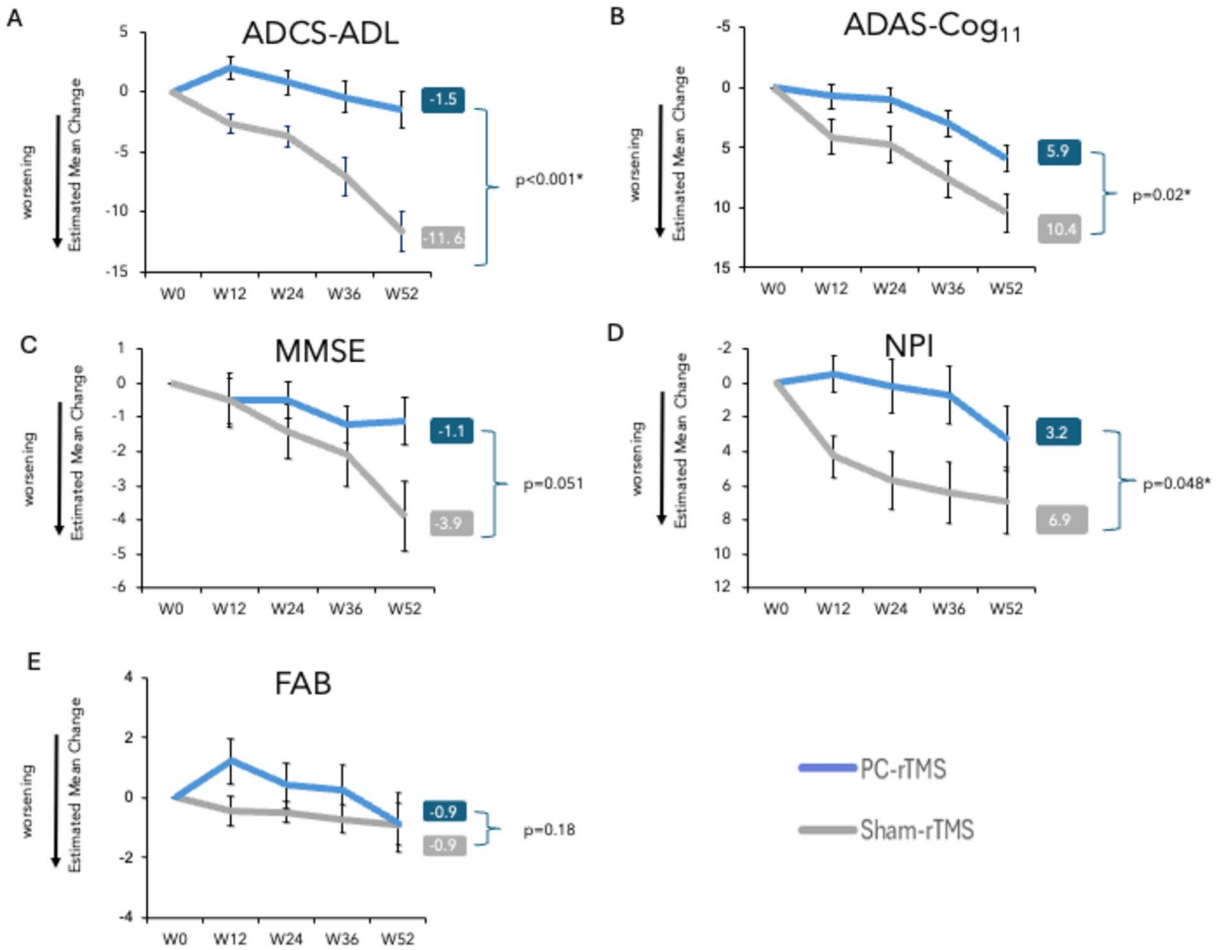

Fig. 3 Secondary outcome measures. Estimated mean group changes for clinical scores. Estimated mean group changes from baseline (W0) in the ADCS-ADL, ADAS-Cog11, MMSE and NPI scores following 12 weeks (W12), 24 weeks (W24) 36 weeks (W36) and 52 weeks (W52) of PC-rTMS and sham-rTMS. Y-axis of each outcome was adapted in order to considering all depicted descending trend as a worsening. **A** GLMM estimated mean score change from baseline for the ADCS-ADL; scores range from 0 to 78, with lower scores indicating worse function. **B** GLMM estimated mean score change from baseline for the ADAS-Cog11; scores range from 0 to 70, with higher scores indicating worse cognition. **C** GLMM estimated mean score change from baseline on the MMSE; scores range from 0 to 30, with lower scores indicating worse cognition. **D**. GLMM mean score change from baseline on the NPI; scores range from 0 to 144, with higher scores indicating worse behavioural symptoms. **E**. GLMM mean score change from baseline on the FBI; scores range from 0 to 18, with higher scores indicating better cognitive performance. Baseline is plotted at Week 0, which is the baseline measurement before the first rTMS session. Error bars indicate standard errors

we observed significant changes on appetite disturbance, apathy and euphoria ($p < 0.05$) (Table 4).

GLMM on FAB scores did not show any significant main effect nor interaction (all ps > 0.05) (Fig. 3E).

Figure 4A depicts the TEPs recorded at W0 and W52 in the PC-rTMS and in the sham-rTMS group and their reconstruction in source space. TEPs consisted in a well-known sequence of positive and negative deflections lasting around 150 ms after the TMS pulse, as expected [27–30]. GLMM on TEP amplitude did not reveal any significant effect (all ps > 0.05). The source propagation analysis showed a larger diffusion in fronto-medial areas at 52W in the PC-rTMS group, although, also in this case, GLMM on source propagation did not reveal any significant effect (all ps > 0.05). GLMM on EEG spectral power did not reveal any significant effect (all ps > 0.05). Analysis of linear relations between the primary outcome change at W52 and the TMS-evoked source propagation over fronto-medial areas examined at W0 revealed a significant correlation when considering the DMN signal propagation of the PC-rTMS group ($r = -0.594$; $p = 0.02$) but not of the sham-rTMS groups ($r = -0.247$; $p = 0.41$) (Fig. 4B).

Table 4 W52-W0 score difference for each NPI subitem in the rTMS group

NPI subitem	t	p	Mean difference	SE difference	d
delusions	0.379	0.646	0.222	0.586	0.135
allucinations	0.628	0.733	0.095	0.152	0.224
agitation	0.943	0.823	0.754	0.799	0.336
depression	-0.573	0.286	-0.333	0.582	-0.204
anxiety	-0.926	0.181	-0.373	0.403	-0.33
euphory	-1.706	0.049*	-0.437	0.256	-0.608
apathy	-1.788	0.042*	-1.373	0.768	-0.637
disnihibition	-0.191	0.425	-0.159	0.831	-0.068
lability	-0.601	0.276	-0.532	0.885	-0.214
aberrant motor	0.19	0.575	0.151	0.792	0.068
sleep	0.777	0.778	0.381	0.491	0.277
appetite	-1.913	0.033*	-1.516	0.792	-0.682

* indicate $p < 0.05$

Discussion

Here we present the results of a 52-week personalized treatment with rTMS of the PC in patients with mild-to-moderate AD. To the best of our knowledge, this is one of the longest trials of brain stimulation to evaluate the safety and effectiveness of rTMS compared to a sham treatment over a long-term interval of 52 weeks. Overall, our results indicate that PC-rTMS is safe and well tolerated by AD patients. Adverse events were rare and mild, consistent with findings from other randomized controlled trials using rTMS in patients with comparable disease severity.

52 weeks of rTMS of the PC resulted in beneficial effects for the primary clinical outcome, which was the change in the CDR-SB score. AD patients treated with PC-rTMS showed a reduced decline in the CDR-SB score as compared to sham of 52%. Positive effects of PC-rTMS on cognitive functions and functional abilities were also confirmed by the analysis of key secondary outcome measures (ADAS-COG11, MMSE, ADCS-ADL, NPI). The most striking effects were observed for the patients' functional decline. AD patients treated with PC-rTMS showed a reduced decline in the ADCS-ADL score as compared to sham of 96%. Hence, patients treated with rTMS did not vary their autonomies of daily living after 52 weeks of treatment, as revealed by the ADCS-ADL score, suggesting a potential use of PC-rTMS in maintaining functional impairments unvaried in the early stages of AD. This result is especially relevant when considering the increased caregiver burden associated with the reduction of ADCS-ADL observed in the sham-rTMS group.

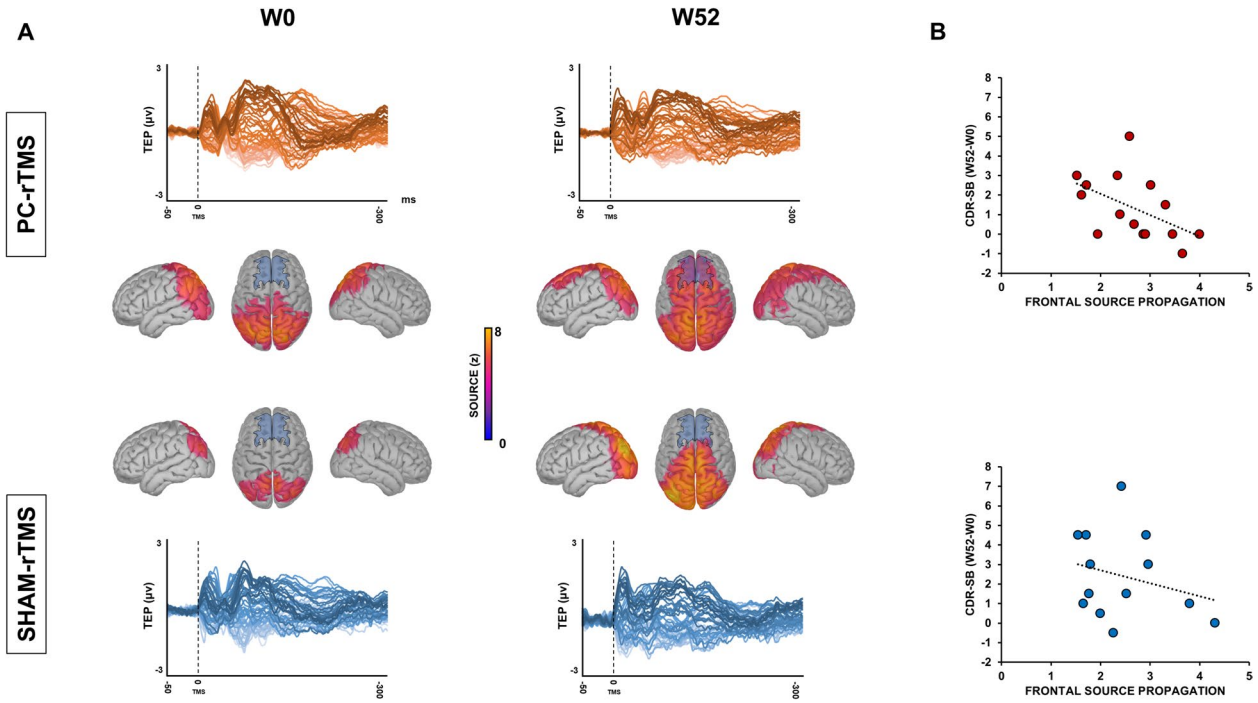

Fig. 4 TMS-EEG source analysis. **A** TEPs were recorded using TMS-EEG applied over the precuneus using neuronavigation system. **B** TEPs and source analysis before (W0) and after 52 weeks (W52) of PC-rTMS (red lines, top plots) and sham-rTMS (blue lines, bottom plots). We did not observe any significant change between the two groups. **C** Correlation analysis between the CDR-SB individual score change from baseline (W52-W0) and the baseline source activity propagation in the PC-rTMS group (red dots, top) and in the sham-rTMS group (blue dots, bottom)

Another important result was the positive effects on behavioral disturbances measured by the NPI scale. We found that beneficial effects were observed in some NPI sub-scales including apathy, euphoria and eating disorders. In patients with AD, simultaneous application of rTMS and tDCS over the bilateral angular gyrus resulted in greater improvement in apathy than sham [31]. This new potential application of NIBS is of great interest since there are no effective pharmacological interventions for apathy in AD or MCI and apathy can have detrimental effects on patient quality of life, dementia severity, disease progression, and caregiver burden [32].

Notably of 48 patients initially enrolled only 32 were able to accomplish the entire trial duration. This was mainly due to COVID pandemic that impeded weekly access to rTMS treatment in many patients, reducing remarkably the number of observations analyzed in the statistics.

We argue that the positive effects induced by rTMS may be ascribed to the impact on cortical plasticity mechanisms, which are known to be impaired in AD at the early disease stages [33]. The impairment of long-term potentiation-like (LTP-like) cortical plasticity has been recently identified as one of the key neurophysiological features in AD [9]. From this perspective, rTMS might be an ideal tool to restore altered LTP and promote functional rearrangements of synaptic activity. Experimental studies in animal models of AD have shown that rTMS restores key neurophysiological mechanisms related to synaptic functions such as LTP and ion channel activity. rTMS rescued deficits in LTP and spatial memory of rats with Aβ-injection, indicating that rTMS noninvasively and effectively increases hippocampal neurotrophins and NMDA-receptor contents in Aβ(1–42)-induced toxicity model rats [34]. Moreover, rTMS counteracts the reduction in neuronal excitability and ion channel activity in dentate gyrus granule neurons, as demonstrated by patch clamp recordings [35]. Other studies confirmed that rTMS decreases Aβ and phosphorylated tau deposits, increases neurogenic proteins such as brain-derived neurotrophic factor, and reduces pro-inflammatory cytokines such as IL-6 and TNF-α [10, 11]. In animal models of AD, rTMS treatment inhibits the expression of BACE1 and elevates the level of IDE, suggesting that the reduction of Aβ load could be attributed to the inhibition of Aβ production and facilitation of Aβ degradation [36]. Importantly, rTMS treatment significantly increased the drainage efficiency of brain clearance pathways, including the glymphatic system in brain parenchyma and the meningeal lymphatics, in the 5xFAD mouse model [37]. On the basis of this background and of the current evidence rTMS might be considered as a new promising therapeutic approach that could be used in AD patients not only per se but also in combination with novel drugs acting on different mechanisms of action such as anti-amyloid anti-tau, and neural inflammation [38].

The current study indeed provides support to the hypothesis that longer period of stimulation (i.e. 52 weeks as compared to 24 weeks) may lead to more durable and sustained effects, as also showed in other studies [39]. How much these effects can last after suspension of treatment needs to be clarified in future studies.

When analyzing neurophysiological TMS-EEG and quantitative EEG measurements we did not found any significant effect, as opposite to the data collected in our recent 24 weeks trial [3] in which we found that TEPs amplitude and gamma oscillations differed between the experimental groups. We believe that the lack of statistical effects is due to the relatively small number of patients that terminated the study and were analyzed at week 52. However, we found that stronger DMN connectivity at baseline measured by TMS-EEG source analyses reconstruction was associated with favorable response to rTMS treatment. This is consistent with the notion that the integrity of DMN is key for maintaining cognitive and functional integrity in patients with AD. This is in line with what we observed in the propagation of TMS-evoked source activity, that seems to be stronger, although not statistically significant, after 52 weeks of PC-rTMS. Future studies need to assess the potential of TMS-EEG measures of DMN connectivity in representing a useful biomarker to select patients that might respond better to rTMS treatment in future studies or clinical applications.

Limitations

The current study has some important limitations. First, more than half of the patients were enrolled in the study agreeing to participate to an extension of a previous study with the same characteristics in which they were initially asked to be treated with rTMS or sham for 24 weeks [3]. Hence this could be a potential confound for the validity of the current results since not every patient agreed to participate to a long period of stimulation of 52 weeks since the beginning of the rTMS or sham treatment. This is a relevant limitation of the study and may have introduced a source of bias related to the study design. Second, our data were collected in the context of a single site trial with a modest sample size. Moreover, the trail was affected by COVID pandemics, and many patients were lost in course of treatment, being not able to continue weekly rTMS therapy and thereby limiting the statistical impact of the current data. Fourth, we did not monitor here CSF or blood-based biomarkers providing evidence

for the biological effects of rTMS that should be provided in future confirmatory trials.

Conclusions

PC-rTMS may reduce the progression of cognitive decline and delay the impairment of autonomies of daily living and behavioural disturbances. Further personalization development [40] and longer treatment interventions might pave the way to a novel class of non-pharmacological intervention for AD.

Abbreviations

FAD	Familiar Alzheimer's disease
AD	Alzheimer's disease
ADAS-Cog	Alzheimer's Disease Assessment Scale– Cognitive Subscale
ADCS-ADL	Alzheimer's Disease Cooperative Study–Activities of Daily Living scale
AEs	Adverse events
APOE	Apolipoprotein E
Aβ	Amiloyd beta
BACE1	Beta-site amyloid precursor protein cleaving enzyme 1
CDR	Clinical Dementia Rating
CDR-SB	Clinical Dementia Rating Scale Sum of Boxes
COVID	Coronavirus disease
CSF	Cerebrospinal fluid
DMN	Default Mode Network
EEG	Electroencephalography
FAB	Frontal Assessment Battery);
fMRI	Functional magnetic resonance imaging
GLMM	Generalized linear mixed models
IDE	Integrated development environment
IL-6	Interleukin 6
LTP-like	Long-term potentiation-like
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MNI	Montreal Neurological Institute
NIBS	Non-invasive brain stimulation
NMDA	N-Methyl-D-aspartate
NPI	Neuropsychiatric Inventory
PA	Posterior-anterior
PC	Precuneus
PET	Positron Emission Tomography
RAVLT	Rey Auditory Verbal Learning Task
rTMS	Repetitive transcranial magnetic stimulation
SD	Standard deviation
tDCS	Transcranial direct current stimulation
TEPs	TMS-evoked potentials
TMS	Transcranial magnetic stimulation
TNF-α	Tumor necrosis factor alpha

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-025-01709-7.

Supplementary Material 1.

Acknowledgements

The authors would like to gratefully acknowledge the volunteer participants, the caregivers, and families for their participation in the trial.

Authors' contributions

GK, AM, ES and MB participated in the study concept and design. SB, EPC, IB, MA, MM, FDL, RE, CM, VP, MF, AD, LM and MM contributed to study execution. GK prepared the first draft of the manuscript. EPC performed the statistical and neurophysiological analysis, and all authors participated in the interpretation of the data. All authors critically reviewed the paper for important intellectual content, participated in revisions, and approved the submitted version. GK and AM had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

The trial was supported by grants of the Italian Ministry of Health to GK (grant n° GR-2016–02364718); the BrightFocus Foundation to GK (A2019523S) and the Italian Ministry of University and Research under the National Recovery and Resilience Plan to EPC (Fondi DM 502/2022 – PNRR MC42 Bando Giovani Ricercatori; MSCA_0000086_FIS01).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was conducted in a research hospital in Italy (Santa Lucia Foundation IRCCS). The trial was approved by the review board and the local ethics committee of the Santa Lucia Foundation IRCCS (Prot. CE/Prog.716) in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. All patients or their relatives or legal representatives provided written informed consent. Patients could withdraw at any point without prejudice. This report followed the CONSORT reporting guideline for randomized studies. The study was registered on the clinicaltrial.gov website on 07–07-2022 (NCT05454540). An independent committee monitored the patients' safety according to the Data Monitoring Committee Charter.

Consent for publication

Not applicable.

Competing interests

GK is scientific co-founder and holds stocks of Sinaptica Therapeutics. GK has received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from: Epitech, Roche, Novo Nordisk. GK and AM have the following patent issued: Combination drug formulations including rotigotine and an acetylcholinesterase inhibitor for the treatment of neurodegenerative diseases (n. 20230381512); GK and ES have the following patent issued: Systems and methods for providing personalized targeted non-invasive stimulation to a brain network (n. 11998740). GK reports grants from Epitech, Alzheimer's Drug Discovery Foundation, Italian Ministry of Health and non-financial support from UCB Pharma outside the submitted work. ES is is scientific co-founder and holds stocks of Sinaptica Therapeutics. AM reports grants from Alzheimer's Drug Discovery Foundation, Italian Ministry of Health and non-financial support from UCB Pharma outside the submitted work.

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Received: 5 December 2024 Accepted: 4 March 2025
Published online: 02 April 2025

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