Received: 25 September 2020 | Accepted: 18 October 2022

DOI: 10.1111/jnp.12299

the british psychological society
promoting excellence in psychology

REGISTERED REPORT STAGE 2

Episodic memory effects of gamma frequency precuneus transcranial magnetic stimulation in Alzheimer's disease: A randomized multiple baseline study

Artemis Traikapi[1] | **Ioanna Kalli**[1] | **Andrea Kyriakou**[1] | **Elena Stylianou**[1] | **Rafaella Tereza Symeou**[1] | **Akrivi Kardama**[2] | **Yiolanda Panayiota Christou**[3] | **Phivos Phylactou**[1] | **Nikos Konstantinou**[1]

[1]Department of Rehabilitation Sciences, Faculty of Health Sciences, Cyprus University of Technology, Limassol, Cyprus

[2]Rehabilitation Center Melathron Agoniston EOKA, Limassol, Cyprus

[3]Acropoleos Medical Center, Nicosia, Cyprus

Correspondence

Artemis Traikapi, Department of Rehabilitation Sciences, Faculty of Health Sciences, Cyprus University of Technology, Vragadinou 15, Limassol 3041, Cyprus.
Email: ag.traikapi@edu.cut.ac.cy

Abstract

Episodic memory decline is the prominent neuropsychological feature of typical Alzheimer's Disease (AD), for which current treatments have a limited clinical response. Recently, gamma entrainment therapy has been used as a non-invasive treatment in AD, providing evidence that it may have the potential to alleviate brain pathology and improve cognitive function in AD patients. At the same time, the precuneus (PC) has been recognized as a key area involved in AD related memory deficits and as a key node of the Default Mode Network. This study aimed to investigate the effectiveness of a 40 Hz Transcranial Magnetic Stimulation (TMS) intervention, delivered bilaterally to the precuneus for 10 days, in improving the patients' episodic memory performance. Secondary outcome variables investigated included general cognitive function, semantic and spatial memory, as well as attention and executive function. A concurrent multiple baseline design across five cases was employed. Four patients completed the study. Visual analysis combined with effect size indices were used to evaluate changes across phases. An increase in the average level of immediate recalled words was observed in three out of four patients. Effect size indices indicated significant improvement of attention skills in two patients. No treatment effect was observed for semantic and visual memory, or for executive function. An

immediate treatment effect was observed in all patients' general cognitive function as assessed with the Alzheimer's Disease Assessment Scale (mean reduction of 5 points), which was maintained and improved further three months post-treatment. The neuropsychological evaluations indicated improved performance three months post-treatment in immediate and delayed recall, attention, phonological verbal fluency, anxiety, and neuropsychiatric symptoms. This study provides preliminary evidence for the efficacy of a novel non-pharmacological treatment using gamma-band TMS in addressing cognitive dysfunction in AD.

KEYWORDS
Alzheimer's Disease, episodic memory, gamma frequency brain stimulation, precuneus, transcranial magnetic stimulation

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the presence of amyloid aggregations (Aβ) and neurofibrillary tangles (tau pathology; Nestor et al., 2004). Tau pathology is prominent in the medial temporal lobe causing profound cell loss and synapses in specific cortical layers in the hippocampal formation, early in the disease (Braak et al., 1993, 2006; Mattsson et al., 2019; Price et al., 1991). Consistent with this pathology, episodic memory decline, such as inability to learn and recall new information, is the hallmark neuropsychological feature of preclinical AD and the best discriminator between healthy individuals and AD patients (Bäckman et al., 2001; Petersen et al., 1994).

In addition to the role of hippocampal cortex degeneration in episodic memory deficits (Gallagher & Koh, 2011), recent neuroimaging studies suggest that aberrant neural activity exacerbates AD's pathology and ultimately disrupts neural networks involved in memory and general cognition of AD patients (Canter et al., 2016). For instance, it has been well established that the default mode network (DMN), which plays a key role in episodic memory and in higher cognitive functions (Buckner et al., 2008) is highly affected in AD, by way of connectivity disfunctions between several important nodes, such as the precuneus (PC), the parahippocampal gyrus and the posterior cingulate gyrus (PCC; Zhou et al., 2015). In addition to that, gamma frequency oscillation abnormalities have been identified in AD and these result in reduction of anatomical connectivity and may account for some of the AD related cognitive impairments (Uhlhaas & Singer, 2006).

Over the recent years, researchers have begun to explore the pathogenesis of AD from different perspectives. One of them suggests that the restoration of gamma oscillations may be the key to treating the disorder's pathology and improve cognition (Fan et al., 2020). For example, Goutagny et al. (2013) indicated that the reduction of slow gamma activity (25–50 Hz) in the hippocampal area CA1 of a mouse model of AD can result in impaired memory function. Moreover, three mice studies demonstrated that gamma stimulation ameliorates AD pathology and cognitive impairment (Adaikkan et al., 2019; Iaccarino et al., 2016; Martorell et al., 2019). Specifically, gamma stimulation by light and sound in a mouse model of the disease, led to the reduction of the two hallmarks of AD pathology, the amyloid-β and the tau protein, while it increased the gene expression of microglia cells, which are responsible for the disposal of harmful clutter in the brain such as the amyloid-β. These findings raised the interesting question of whether an increase in gamma frequency neural activity in specific brain areas in humans, can restore the cognitive deficits resulted from AD.

Transcranial Magnetic Stimulation (TMS) has been investigated, over the last decades, as a non-invasive technique for brain stimulation. Over the last two decades TMS has been used as a non-pharmacological approach in AD, providing evidence that it can significantly improve cognition (Rabey et al., 2013; Zhao et al., 2017). To date, the most commonly used TMS protocol in AD is a high frequency stimulation at 20 Hz, targeting the dorsolateral prefrontal cortex. This research has demonstrated improvements in patients' sentence comprehension (Cotelli et al., 2011), global cognitive function (as measured with the Mini Mental State Examination and the Alzheimer's Disease Assessment Scale) and behavioural symptoms like anxiety and fear (Ahmed et al., 2012; Yue et al., 2015). Yet, it remains unclear, whether TMS can improve episodic memory function following high frequency gamma stimulation over brain areas previously shown to be implicated in episodic memory, such as the PC.

Functional imaging data on brain activation patterns during episodic memory tasks suggest strong involvement of the PC in most episodic memory tasks (Krause et al., 1999; Rugg et al., 2002; Schmidt et al., 2002; Shallice et al., 1994). PC activation is evident during the classic laboratory episodic memory tasks (*e.g.*, word lists or pared association tasks), but importantly it is also observed in naturally acquired autobiographical memories (Gilboa et al., 2004; Lundstrom et al., 2005). Evidently, AD patients exhibit cortical atrophy and abnormal activity in PC during memory tasks (Chen et al., 2017). At the same time, impaired functional connectivity between bilateral PC and hippocampus is also evident in the early AD stages (Kim et al., 2013; Sorg et al., 2007). Finally, the PC/PCC node is considered particularly significant as it is the only node that directly interacts with all the DMN cortical structures (Fransson & Marrelec, 2008). Recent evidence suggests that application of repetitive TMS on key DMN nodes, such as the PC, improves episodic memory performance in healthy controls (Bonnì et al., 2015; Rose et al., 2016). Therefore, stimulation of the PC using TMS may improve the disrupted connectivity between the PC and the hippocampus, as well as the abnormal functional connectivity between PC and other important nodes of the DMN, hence to effectively reverse AD related cognitive decline.

To our knowledge, up to date only one study has investigated the effect of high frequency stimulation over the PC in AD (Koch et al., 2018). In this study, TMS at 20 Hz was applied bilaterally to the PC demonstrating a significant effect in episodic memory recall. In addition, TMS induced neural activity modulation in PC and medial frontal cortex, suggesting relevant modulation over the medial parieto-frontal circuit. Finally, TMS at 20 Hz prompted an enhancement of beta activity over the PC. These findings provide the first evidence that the PC may be a novel stimulation target to enhance the affected networks and improve patients' memory performance. However, the effect that gamma frequency stimulation will have on the disrupted gamma oscillations documented in AD, and hence to memory performance, remains to be seen.

The objective of this study was to investigate whether gamma frequency stimulation of the PC has the potential to improve episodic memory function in mild to moderate AD. We applied gamma frequency stimulation at 40 Hz, using TMS to the PC and investigated whether it has an effect on the patient's performance on immediate and delayed wordlist recall tests. The wordlist tests were based on the Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-cog), and on word lists designed for the study (see Method).

We hypothesized that gamma stimulation applied bilaterally to the PC will significantly improve the patients' performance on episodic memory tasks. Moreover, given that TMS on the PC generates effects not only at the stimulation site but at network level too (Koch et al., 2018; Mancini et al., 2017), we hypothesized that our proposed treatment protocol will enhance the disrupted gamma activity and brain connectivity and hence, will have a wider positive effect on patient's cognitive abilities. Specifically, we expected improvements on: (1) patient's global cognition, assessed by the ADAS-cog; (2) semantic memory, assessed by semantic association and a naming task; (3) spatial memory, assessed by a corsi block task and, (4) attention and executive functions, assessed by the Trail Making Test.

METHODS

Study design

This study is a pre-registered report. The approved stage 1 protocol can be found here: https://osf.io/5yhuv/.

We employed a single case, randomized, concurrent multiple baseline design, across five participants (Hayes et al., 1999). This design involves multiple AB series (A = baseline, B = intervention), in which the baseline phases begin at the same time for each participant, while the intervention is introduced staggered across time and participants. The staggered introduction of the intervention allows to demonstrate that the targeted behaviours do not change over time, but only after the introduction of the treatment (Lobo et al., 2017).

The study was designed, conducted, and reported according to the 'What Works Clearinghouse' criteria for single case studies (Kratochwill et al., 2010). According to these criteria, to meet 'evidence standards' a minimum of three AB series repetitions (three participants) with at least three measurements of the outcome variables on each phase is recommended. In addition, to minimize major threats of internal and external validity, randomization should be implemented to yield control over confounding variables; the targeted behaviours must be assessed by more than one assessor collecting the necessary inter-assessor agreement, and finally procedures that will ensure that the interventions and the assessments will be delivered as planed (treatment fidelity), are highly recommended (Kratochwill & Levin, 2014). All the above criteria are discussed below. In addition, a schematic representation of the study's design and timeline is illustrated in Figure 1.

The study comprised five AB series repetitions (*i.e.*, each participant represents an AB repetition). The baseline phases comprised five experimental conditions characterized by the length of their periods: one, two, three, four and five-week baseline periods. After the end of each experimental condition patients received a two-week gamma frequency TMS treatment. Participants were randomly allocated to the experimental conditions. With this design the treatment was introduced at different time periods to each participant (*i.e.*, after 1 week to the first participant, after 2 weeks to the second participant *etc.*). The sequential introduction of the intervention makes the participants that stay at the baseline phase the control group. For instance, when the intervention was introduced to the first participant, the remaining patients at the baseline phases served as the control group and hence, no improvements were expected. Accordingly, if the TMS intervention was the sole determinant of improvement, no changes to the targeted behaviours would be expected for the participants reimaging in the baseline phase. Data were obtained at pre-treatment, baseline, treatment, post-treatment and after a three-month follow up period.

Single case data collection

Baseline phases

Five baseline phases of different length were implemented (*i.e.*, 1–5 weeks). The targeted behaviours were evaluated two times per week except for the participant in the one-week condition who was evaluated three times in order to meet evidence standards, which require at least three assessments on each phase. Therefore, the patient in the one-week baseline was evaluated three times, the patient in the two-week baseline condition was evaluated four times, the patient in the three-week condition six times *etc.* (see Figure 1). Each assessment session lasted between 30 and 40 minutes.

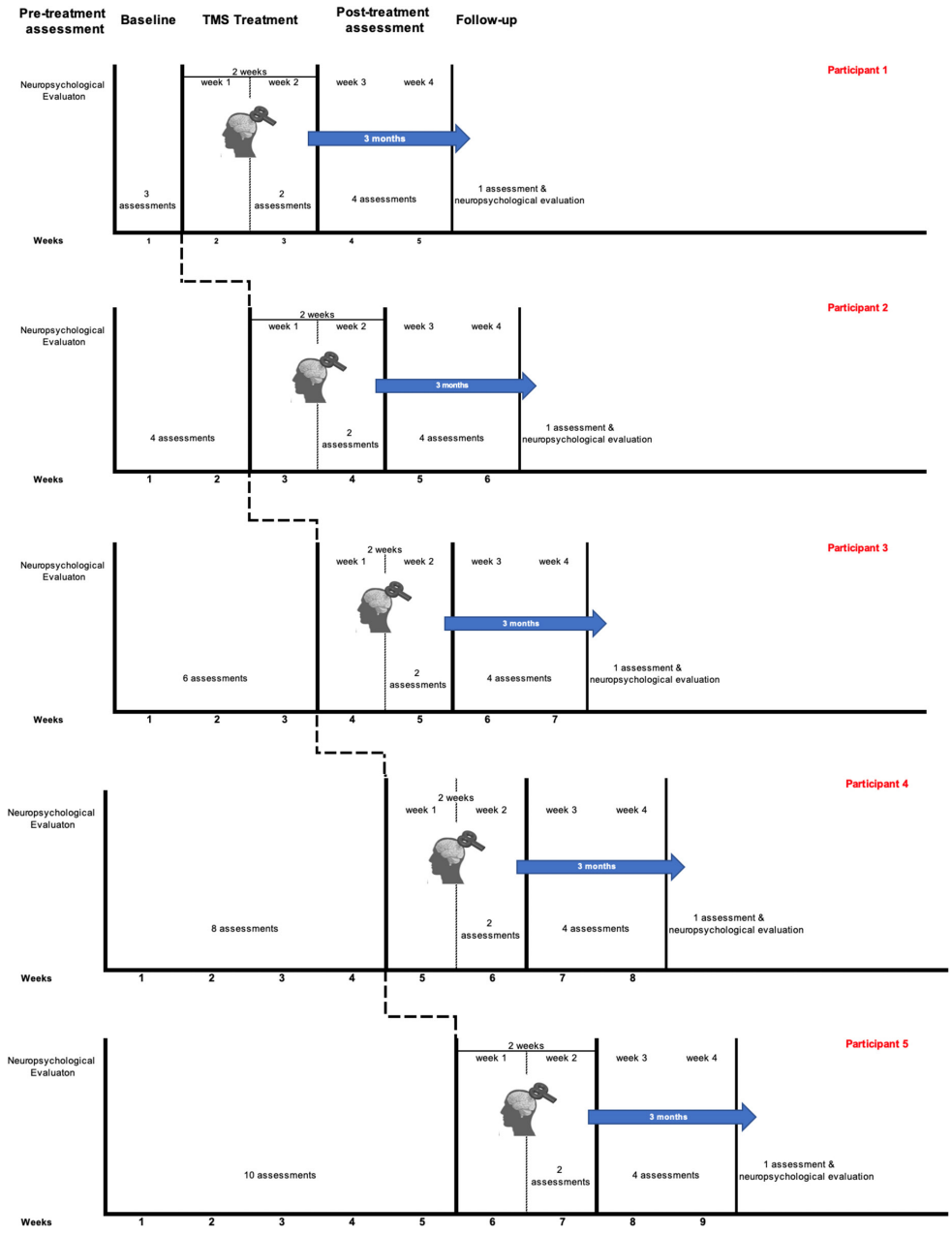

FIGURE 1 Schematic representation of study's design and timeline

Intervention phases

Participants underwent a two-week TMS intervention immediately after the end of their experimental baseline condition. The TMS sessions were being delivered daily (Monday to Friday) for a total of 10 TMS sessions. The targeted behaviours were assessed six times in total. As a delayed effect was expected from the TMS treatment (*e.g.*, Cotelli et al., 2011), the data collection started one week after the beginning

of the intervention. Therefore, two assessments were conducted during the treatment phase and four post-treatment (two assessments/week; see Figure 1).

Follow-up phase

The targeted behaviours were assessed again three months after the end of the intervention phases.

Participants – Inclusion/Exclusion criteria

Five patients with a diagnosis of probable Alzheimer's Disease were recruited for this study. A diagnostic protocol was implemented to verify the presence of Alzheimer's Disease and eliminate other possible conditions that may induce dementia. The diagnosis was given by a certified neurologist according to the NIA-AA criteria (McKhann et al., 2011). To participate in the study participants had to meet all the following criteria: having a Mini Mental State Examination (MMSE) score between 17 and 24, 4 or 5 on the Global Deterioration Scale, between 10 and 20 on the Greek version of the Instrumental Activities of Daily Living (IADL) and no less than 5 in the Basic Activities of Daily Living (BADL). Participants had to have a stable medical and pharmacological condition for at least 2 months prior to the study, be free from any clinically significant medical history that may induce cognitive deterioration (psychiatric, neurological, cerebrovascular), their visual and hearing abilities to be within the normal range, be willing to undergo an MRI scan and to have a caregiver who was agreed to be responsible for their participation throughout the study.

Patients with history of excessive alcohol consumption or under psychoactive medication within the past 2 months were excluded from the study. Patients with diagnosis of epilepsy or family history of epilepsy; with moderate or severe depression, as assessed by the Geriatric Depression Scale – 30 (score no more than 15); with severe loss of hearing or visual ability as was identified during the initial interview; with medical implants in the head or a pacemaker; with history of brain injury, surgery to the heart or stroke; under drugs with anticholinergic properties and without a caregiver who could take the responsibility for their commuting throughout the study, were also excluded.

TMS: Sites, protocol, and procedure

In each B phase (*i.e.*, intervention phase) patients received daily treatment sessions for 2 weeks (one session per day; five sessions per week; total of 10 sessions). Each session included 25 trains consisting of 1 s of 40 Hz each (40 pulses/train; 1000 total pulses), with 29 sec inter-train intervals and delivered at 90% of participant's resting motor threshold, or with the maximum intensity of 65% of the maximal machine output (for safety reasons) using the Magstim Super Rapid^2 Plus^1 Therapy System with a figure-of-eight coil. Coil was oriented parallel to the midline with the handle pointing down-ward. Motor threshold (MT) was evaluated at the begging of each treatment week (two times total) and TMS intensity was adjusted accordingly. MT was defined as the minimum TMS intensity needed to elicit MEPs of >50 μV in five out of 10 trials in the first dorsal intereosseous muscle.

Stimulation was delivered over the left and right precuneus on separate days (*i.e.*, one day left precuneus and the contralateral precuneus the following day). The exact stimulation sites were identified by targeting at the peak voxels that have been previously reported to be activated during episodic memory tasks. Therefore, the left PC located at the MNI coordinates $x = -14$, $y = -66$, $z = 56$ (Hebscher et al., 2020) and the right precuneus at $x = 6$, $y = -70$, $z = 44$ (Kwok et al., 2012; Ye et al., 2019). The patients underwent an anatomical MRI scan prior to the study and the exact position of the stimulation coil was guided

by the Visor2 TMS neuronavigation system (Visor2, ANT Neuro, Enschede, Netherlands). Prior to the beginning of the TMS sessions, MNI coordinates were inserted into the neuronavigation system and the most superficial regions closest to the coordinates, that could be accessible with TMS, were selected. Due to subjects' anatomical differences, the stimulation sites varied slightly from the initial coordinates.

Outcome measures

The primary measures indicating the patients' episodic memory performance were word learning tasks, with multiple trials. These are the standardized neuropsychological tests for evaluating the ability to learn and recall new information in AD (Zhao et al., 2012). A detailed description is being discussed below. We hypothesized that our proposed TMS protocol delivered to the precuneus will have an effect not only at local, but also at network level (Mancini et al., 2017). Therefore, the secondary outcomes related to measures of general cognition, semantic and spatial memory, as well as attention and executive functions.

Neuropsychological evaluation

Participants underwent a neuropsychological evaluation with standardized and well recognized measures pre-treatment (before the baseline phase) and in the follow up phase. The purpose was to evaluate the effect of our proposed treatment beyond the targeted behaviours (Krasny-Pacini & Evans, 2018). Moreover, the participants' episodic memory abilities were evaluated with more than one cognitive assessment test (word learning test & logical memory). The length of each assessment was approximately 90 minutes.

Single case data

Primary outcomes, during all phases, were assessed using a 'word learning list', which is one of the most well-established tests for assessing episodic memory functioning in patients with early dementia (Beck et al., 2012). Seventeen alternative and equal in difficulty word learning lists were developed. Each list, comprised 5 low, 5 medium and 4 high frequency words. Additionally, the chosen words had similar concreteness and visual imagery ranks, to establish, as much as possible, that all forms had approximately the same level of difficulty. The assessments included 3 learning trials, one delayed recall and a recognition trial. The recognition task of each form, contained the 14 original words and 14 new words equal in difficulty with the original ones.

The secondary outcomes were assessed using: (1) 17 alternative forms of a semantic association task, comprising 15 living and nonliving items, including a verbal and a visuospatial form (Caputi et al., 2016), (2) 17 alternative forms of a naming task, comprised of 15 living and nonliving items, (3) 17 different corsi block tasks, generated using an algorithm which randomly assigned numbers to each form and finally, (4) 17 alternative Trail Making Tests A & B, which were created by maintaining the original position of the cycles (Reitan, 1955) but randomly assigning the numbers and letters in the cycles, which however were maintained in the same half of the page in every alternative form (*e.g.*, the number '1' was in the bottom part of the page on every form). The first form was created for the semantic associations and the naming tasks and then each stimulus was matched with 16 others of similar characteristics (*i.e.*, word frequency, concreteness, and visual imagery) to create the alternative forms. The tests were administered with the same order in all participants. First, the word learning list was given followed by the Trail Making Test, the Naming Test, the Semantic Association Task and finally the Corsi Block Task. The delayed recall and the recognition tack from the word learning list were administered 25 mins after the immediate recall tasks.

ADAS-cog

Finally, the patients were assessed by the standard, 11 items ADAS-cog, including the optional 'delayed recall' item. The ADAS-cog was used pre-treatment, immediate after the end of the treatment and at the follow-up. The ADAS-cog was adjusted and standardized in the Greek-Cypriot population and two alternative forms of equal difficulty were created for the word recall, the word recognition and the object naming subscales. The total scoring ranged between 0 and 80. A drop of three points or more was considered as a positive treatment effect (Schrag et al., 2012).

ANALYSIS

Inter-assessor agreement was calculated by the Percentage Agreement (PA) for each phase on each outcome variable. Minimum acceptable inter-assessor agreement was considered a range between .80 and .90 (Hartmann et al., 2004) on at least 20% of all sessions (Lobo et al., 2017). Two raters, members of the research team, independently marked the scores of 12 assessment forms (*i.e.*, six from the baseline phases and six from the treatment phases) previously obtained by a different assessor. The forms included all the obtained assessment tests.

First stage of analysis

At the first stage of the analysis, to determine whether there was a functional relation between the TMS protocol and the outcome measures, visual analysis was conducted by examining six features of the single case design graphed data: level, trend, stability, immediacy of the effect, overlap, and consistency. Each feature was assessed individually and collectively across phases. When an effect of intervention was evident, the visual analysis was supplemented by randomization tests and effect size indices methods, to evaluate the magnitude of the intervention effect and interpret the results in terms of statistical significance. According to the 'What Works Clearinghouse' single case criteria, three demonstrations of the experimental effect at three different time points, within or across phases, are required to indicate a causal effect between the independent and the dependent variables (Kratochwill et al., 2010).

Visual analysis

The collected data were displayed graphically and a within and between phase examination was performed. *Within-phase examination*: Consistency of level, trend and stability within each phase was examined. The mean score of each variable was used to assess the within phase level, and trend was evaluated by determining whether the data points were decreasing or increasing monotonically. In addition to the visual analysis, quantification indices were used in an attempt to increase internal and external validity. To quantify the within phase differences in level and thus to identify whether there was substantial increase in the targeted behaviours, the Percentage Change Index (PCI) was used. The PCI converts the raw measures into percentages and thus makes the results comparable. Possible changes in the trend of the within phase were estimated by the least squares regression. Within-phase stability (or consistency) was assessed by calculating the percentage of data points, within 15% of the phase mean. Stability criterion was satisfied if 80–90% of data points fall within a 15% range of the mean. In addition, the stability of phases was assessed by examining data variability by constructing standard deviation bands around average levels. *Between-phase examination*. Subsequently, overlap and immediacy of the effect between the baseline phases and the intervention phases as well as the consistency between similar phases were evaluated.

The Percentage of Nonoverlapping Data index (PND) was used to quantify the proportion of data points in the intervention phase that did not overlap with the baseline phase. A PND above 70 was consid-

ered as an indication of effective intervention, a PND between 50–70 was an indicator of a questionable effect, whereas a PND below 50 was considered as no observed effect (Scruggs & Mastropieri, 1998). Immediacy of the effect is usually examined by comparing changes in level between the last three data points of one phase and the three first data points of the next phase. However, TMS protocol was expected to have a delayed effect and therefore the immediacy of effect for the intervention phases was examined using the last three data points instead of the first. Consistency of similar phases was evaluated by examining the extent to which data patterns across baseline and intervention phases were similar (A with A and B with B).

At the final step of visual analysis all information from each phase were combined to determine whether the data across all phases demonstrated at least three indicators of effect at different point times. If the TMS treatment was the sole determinant of improvement, we expected to find indicators of improvement only during the intervention phase.

Second stage of analysis

When a causal relation between the independent and the dependent variable became evident from the visual analysis described above, we next performed randomization tests (RTs). The null hypothesis was that there was no treatment effect from the TMS proposed protocol, therefore participants' responses were independent from the condition (baseline vs. intervention) under which they were observed. The alternative hypothesis was that participants' responses in the outcome measures were improved due to the treatment protocol.

Effect size indices were used as our test statistics (Lobo et al., 2017). Sensitivity analysis was initially planned to be conducted and hence, multiple estimators were scheduled to be calculated, as it is strongly recemented by the What Works Clearinghouse standards. The pre-defined test statistic was planned to be calculated for the Improvement Rate Difference (IRD) and the Nonoverlap of All Pairs (NAP). We planned to construct a randomization distribution and p-value would be calculated for the estimators. We would reject the null hypothesis and accept the alternative hypothesis if the p value was smaller or equal to a = .05. Finally, the Piecewise regression-based approach would be used to calculate the effect size and the consistency across all estimators would be evaluated (Kratochwill et al., 2010).

Treatment fidelity

Treatment fidelity was monitored during the study to ensure that the TMS protocol and the outcome variables assessments were implemented as intended. This allowed us to truly test the effectiveness of our proposed protocol and therefore the danger to commit a Type 1 or Type 2 error was eliminated (Krasny-Pacini & Evans, 2018). A checklist was developed to ensure that all the key elements of our study were implemented as planned. The assessors and the treatment providers underwent training to ensure their skill acquisition. During the intervention sessions, a supervisor continuously observed the procedure to confirm the consistent and accurate administration of the treatment. During the different phases the patients were closely monitored to identify possible variables that could influence the effectiveness of the treatment (*e.g.*, changes in the pharmacological treatment).

RESULTS

Participants

Five patients were recruited for the study and randomly assigned to one of the five baseline conditions. The patient on the week-2 baseline condition withdrew from the study after the first week of treatment sessions. This patient's data were removed from all subsequent analyses. The remaining four patients (see

Table 1) completed the study with 100% treatment adherence. No side effects were reported. After the end of each TMS session, patients were asked to rate, in a skale of 0 (no hurt or disturbance) to 10 (hurts the worst, very disturbing), the feeling of the stimulation (*i.e.*, if they felt pain or any disturbance) during the session. TMS was well tolerated and the patients rated the TMS experience at an average score of 1.5. The stimulation was delivered at 90% of RMT for Patients 1, 2, and 4. Patient 3 was found to have a high RMT (*i.e.*, 81%) and for safety reasons we chose to deliver stimulation at 65% of the machine's output which corresponded to this patient's 80% RMT.

Inter-rated reliability

The analysis indicated a high level of agreement in all the administered neuropsychological tests (PA = 1) during the baseline and treatment phases. A lower, but still acceptable, level of agreement was observed for the naming task (*i.e.*, .92).

Primary outcomes-episodic memory

Immediate word recall

First stage of analysis

The repeated assessments of patients' immediate recall during the A, B and the follow-up phases are illustrated in Figure 2. Phases' characteristics are illustrated in Table 2. The stability criterion was not satisfied within phases. Only Patient 3 demonstrated stable performance during the treatment phase. The percentage of the data points falling within the pre-defined range was between 12.5% to 66.5%, indicating unstable patients' performance during the data points collection in both A and B phases.

The patients' unstable performance during baseline was also evident by the visual inspection of the phases trend lines (see Figure 2). However, an increase in average level was observed for the total number

TABLE 1 Patients' demographic and clinical characteristics

Age (years)	70.75 (5.06)
Sex (% female)	50%
Education (years)	10.75 (5.5)
MMSE	20 (1.8)
BADL	5.87 (.25)
IADL	16.5 (4.9)
GDS	4.25 (.5)
GDS-30	4.75 (4.2)

Note: The table shows the patients' average scores and standard deviations.

Abbreviations: BADL, Basic activities of daily living; GDS, Global Deterioration Scale; GDS-30, Geriatric Depression Scale – 30; IADL, Instrumental Activities of Daily Living; MMSE, Mini Mental State Examination.

FIGURE 2 Visual representation of the measures of immediate recall during the baseline, treatment and follow up phases. *Note*: The vertical lines indicate the start of the following phase. The baseline conditions began at the same time for all participants but the treatment was introduced staggered across time and participants. The black horizontal lines represent the average score per phase. The blue dotted lines illustrate the trend lines for each baseline phase and the treatment + follow up phase. The PCI and PND indices have been calculated without the follow up phase. PCI, Percentage Change Index; PND, Percentage on Nonoverlapping Data.

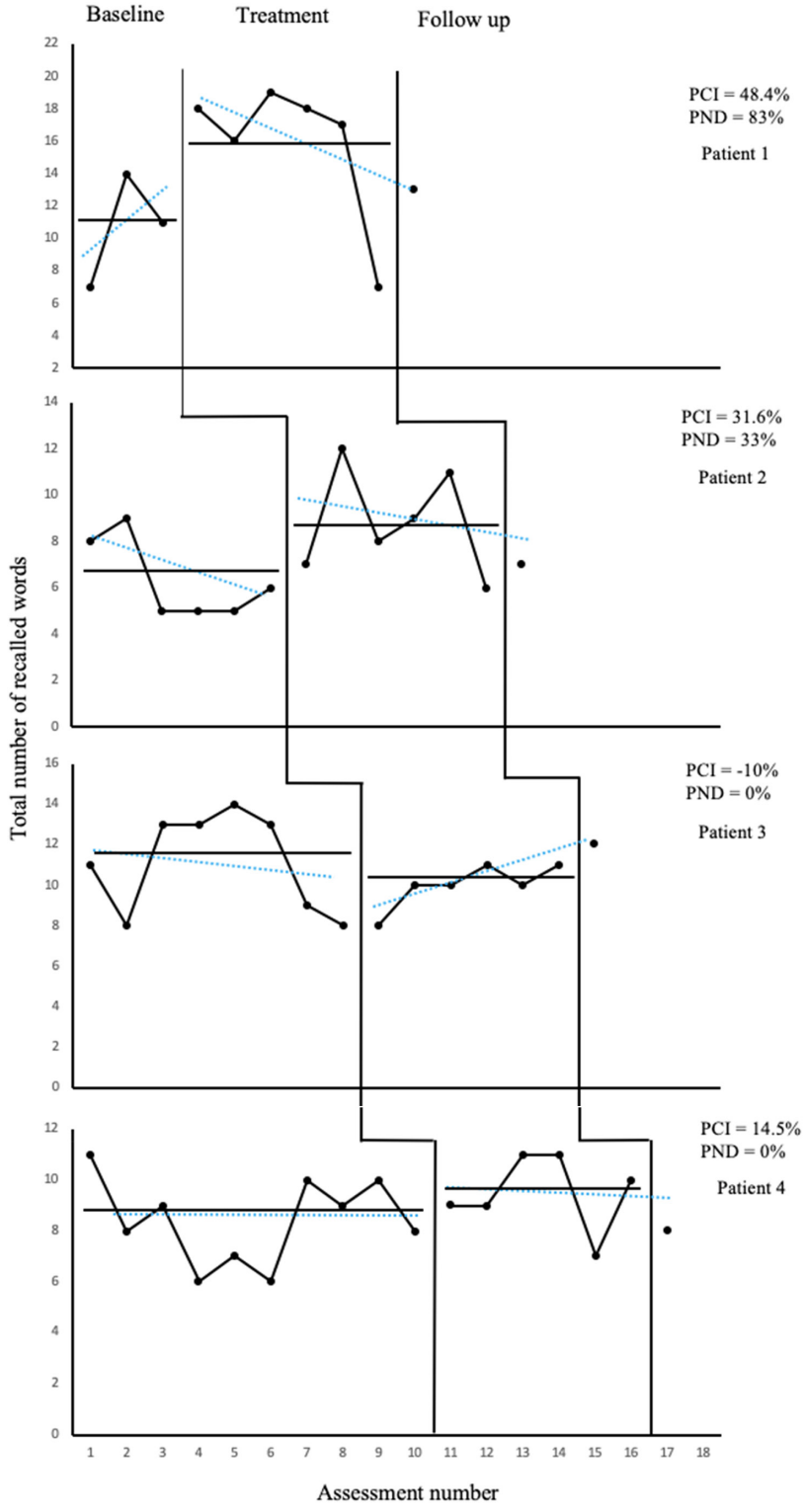

TABLE 2 Phases' characteristics of the immediate recall scores

Patient	Baseline condition (weeks) and total assessments	Number of assessments on the treatment phase	Baseline - Mean (SD)	Treatment - Mean (SD)
1	1 (3)	6	10.7 (3.5)	15.8 (4.4)
2	3 (6)	6	6.3 (1.75)	8.8 (2.3)
3	4 (8)	6	11.1 (2.4)	10 (1.1)
4	5 (10)	6	8.4 (1.7)	9.5 (1.5)

Abbreviation: SD, standard deviation.

of recalled words in the three learning trials, for Patients 1, 2 and, 4 (Figure 2). The PCI signified an increase of 48.4% in the total recalled words after the intervention on Patient 1, 31.6% on Patient 2 and, 14.5% on Patient 4. Patient 3 showed a 10% reduction on the total recalled words after the intervention.

The two standard deviation band around the average levels showed three consecutive data points falling outside the band in Patient 1, indicating an effective treatment effect (Perdices & Tate, 2009). Two, but not consecutive, data points, fell outside the band in Patient 2. No effect was observed in Patients 3 and 4 (Figure 3a).

The PND index indicated a significant effect of the intervention in Patient 1 (PND = 83, $p < .05$; 83% of the data points in the treatment phase did not overlap with the data points from the baseline phase). No observed effect of the treatment was indicated for Patients 2, 3 and 4 (PND = 0, $p > .05$; Figure 2).

Immediacy of the effect was observed in Patients 1 (baseline mean = 10.6 words, treatment mean = 14 words) and 2 (baseline mean = 5.3 words, treatment mean = 8.6 words). Immediate effect was not observed in Patients 3 and 4 (Figure 3b).

Second stage of analysis

While visual analysis provided some indicators of cognitive improvement, failure to establish a consistent pattern during the baseline conditions, prevented reaching safe conclusions for a functional relationship between the treatment and patients' immediate recall performance. The calculated p-value for the NAP estimators did not provide evidence of a significant treatment effect. As the IRD is an insufficient index when strong trend is evident in the baseline conditions (Vannest & Ninci, 2015), it was considered unsuited and was not calculated for the obtained data.

Delayed recall and recognition

Visual analysis did not provide evidence of any improvement in patients' performance after treatment. Phases' characteristics are illustrated in Table 3.

Secondary outcomes

Trail making test A'

First stage of analysis

The patients' repeated assessments during all phases are illustrated in Figure 4. Phases characteristics are illustrated in Table 4. The stability criterion was not satisfied in any phase for all participants, indicating the patients' unstable performance. The highest percentage of data falling within 15% of phase's mean was 50% and the lowest 33%, indicating a high variability in the single case data.

Visual inspection of the phases' trend lines showed highly unstable baseline conditions, making the investigation of a treatment effect difficult. However, visual inspection of the graph data indicated a

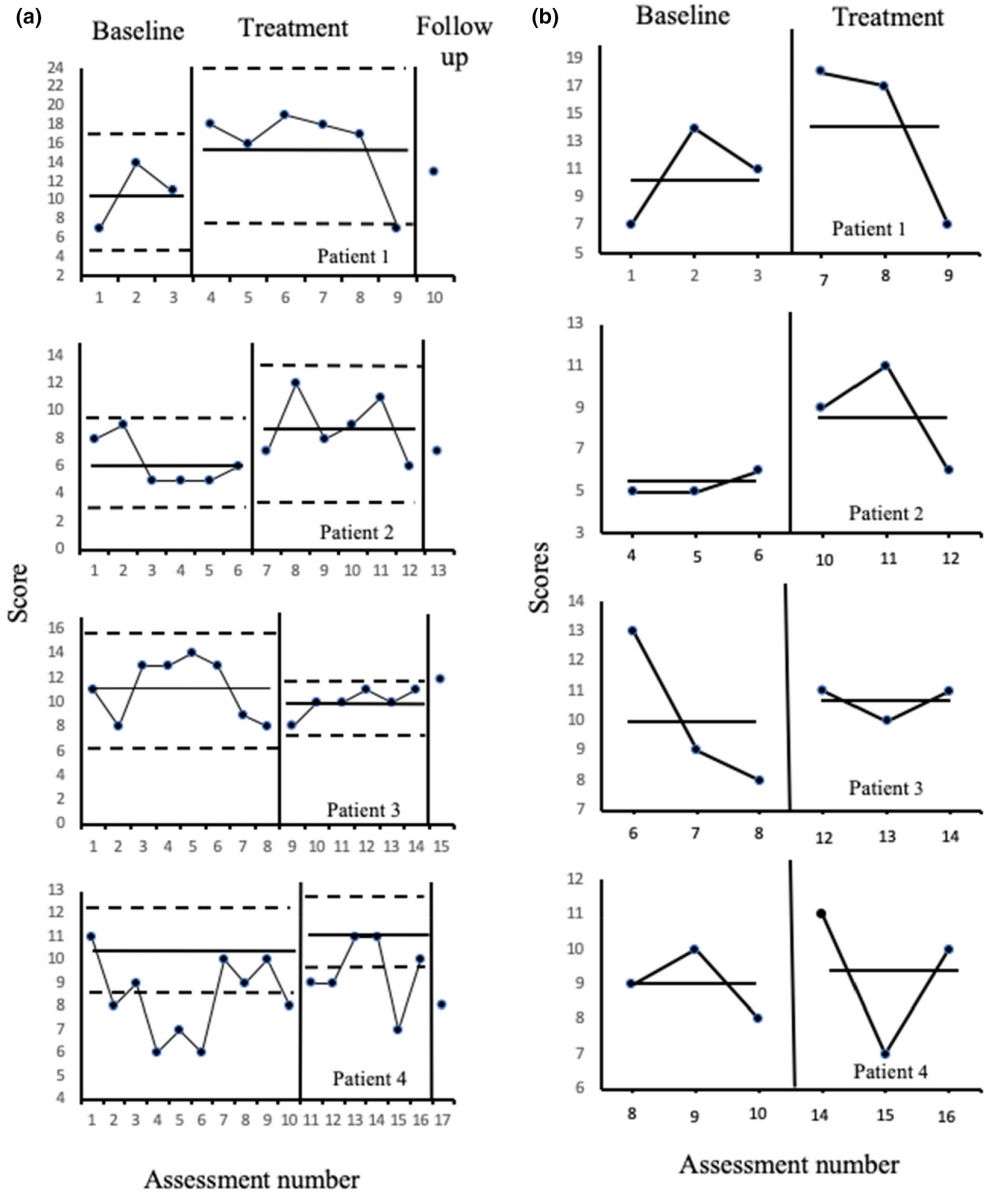

FIGURE 3 Visual analysis of the single case data of the immediate recall assessments.
Note: (a) Two-standard deviation band around the mean. (b) Immediacy of the effect by comparing changes in mean levels of the last three data points of the baseline conditions, with the last three of the treatment conditions.

decrease in the mean levels after the introduction of the intervention compared to the baseline conditions (Figure 4). Each patient's scores refer to the total time in seconds to complete the task, therefore a decreased score after the intervention compared to baseline indicates faster performance which is considered a behavioural improvement due to the intervention. In contrast to the patients' scores during baseline, the PCI showed a decrease of 47.2% in Patient's 1 total time, a decrease of 59.3% in Patient 2 and a decrease of 21.4% in Patient 4. No difference was observed in Patient 3 (PCI = −2.35).

The visual representation of the two standard deviation band around the average levels (Figure 5a) indicated a high variability in the baseline phases. In the baseline condition of Patient 1, where the small-

est variability is observed (SD = 53 seconds) the treatment effect is detected, with three consecutive data points exceeding the band, indicating a significant treatment effect (Perdices & Tate, 2009).

The PND index indicated a significant treatment effect for Patient 1 (PND = 100, $p < .005$) and Patient 2 (PND = 83.3, $p < .005$). Immediacy of the effect was observed in Patient 1 (baseline mean = 259 seconds, treatment mean = 141 seconds) and Patient 2 (baseline mean = 338 seconds, treatment mean = 125 seconds) while Patients 3 and 4 presented increased treatment phase mean (Figure 5b).

Second stage of analysis

The calculated p-value for the NAP estimators did not provide evidence of a significant treatment effect for the Trail Making Test. However, the NAP index was calculated individually for each patient and provided significant results for Patients 1 (NAP = 0, $p < .05$) and 2 (NAP = .03, $p < .05$; Vannest et al., 2016). Due to the observed strong trend during the baseline conditions, the IRD was not calculated for the obtained data (Vannest & Ninci, 2015).

Trail making test B'

The instructions of the test proved too complex for the patients to follow as instructed and we therefore opted to stop data collection of this test.

Semantic association, naming, corsi block tasks

Visual analysis did not provide evidence of differences between the patients' performance before compared to performance after the treatment. Table 5 illustrates phases' characteristics for the semantic associations and the naming tasks and Table 6 illustrates phases' characteristics for the corsi block tasks.

ADAS-cog

All patients presented improvement (*i.e.*, drop in the total score) post-treatment (average score = 28) in relation to their pre-treatment performance (average score = 33; Figure 6a). The effect was slightly higher in the follow-up phase where the average reduction was 5.6 points (average score = 27.4) in relation to the pre-treatment and .6 in relation to the post-treatment (Figure 6b).

TABLE 3 Phases' characteristics for the Delayed Recall and Recognition of the Word Learning Tasks

Patient	Baseline condition (weeks) and total assessments	Number of assessments on the treatment phase	Delayed recall		Recognition	
			BM (SD)	TM (SD)	BM (SD)	TM (SD)
1	1 (3)	6	.33 (.57)	.6 (.8)	5 (0)	6 (1.8)
2	3 (6)	6	0 (0)	.3 (.8)	11.3 (3)	10 (2.3)
3	4 (8)	6	.75 (.75)	1 (0)	6.8 (2.6)	6.1 (2.2)
4	5 (10)	6	.3 (.67)	.16 (.4)	12.9 (2.1)	12.8 (2.4)

Note: The score on the recognition task refers to the total number of words that were not recognized correctly.

Abbreviation: SD, standard deviation.

FIGURE 4 Visual representation of the obtained single case data for the Trail Making Test A' during the baseline, treatment and follow up phases.

Note: The vertical lines indicate the start of the following phase. The black horizontal lines represent the average score per phase. The blue dotted lines illustrate the trend lines for each baseline phase and the treatment + follow up phase. PCI, Percentage Change Index; PND, Percentage on Nonoverlapping Data.

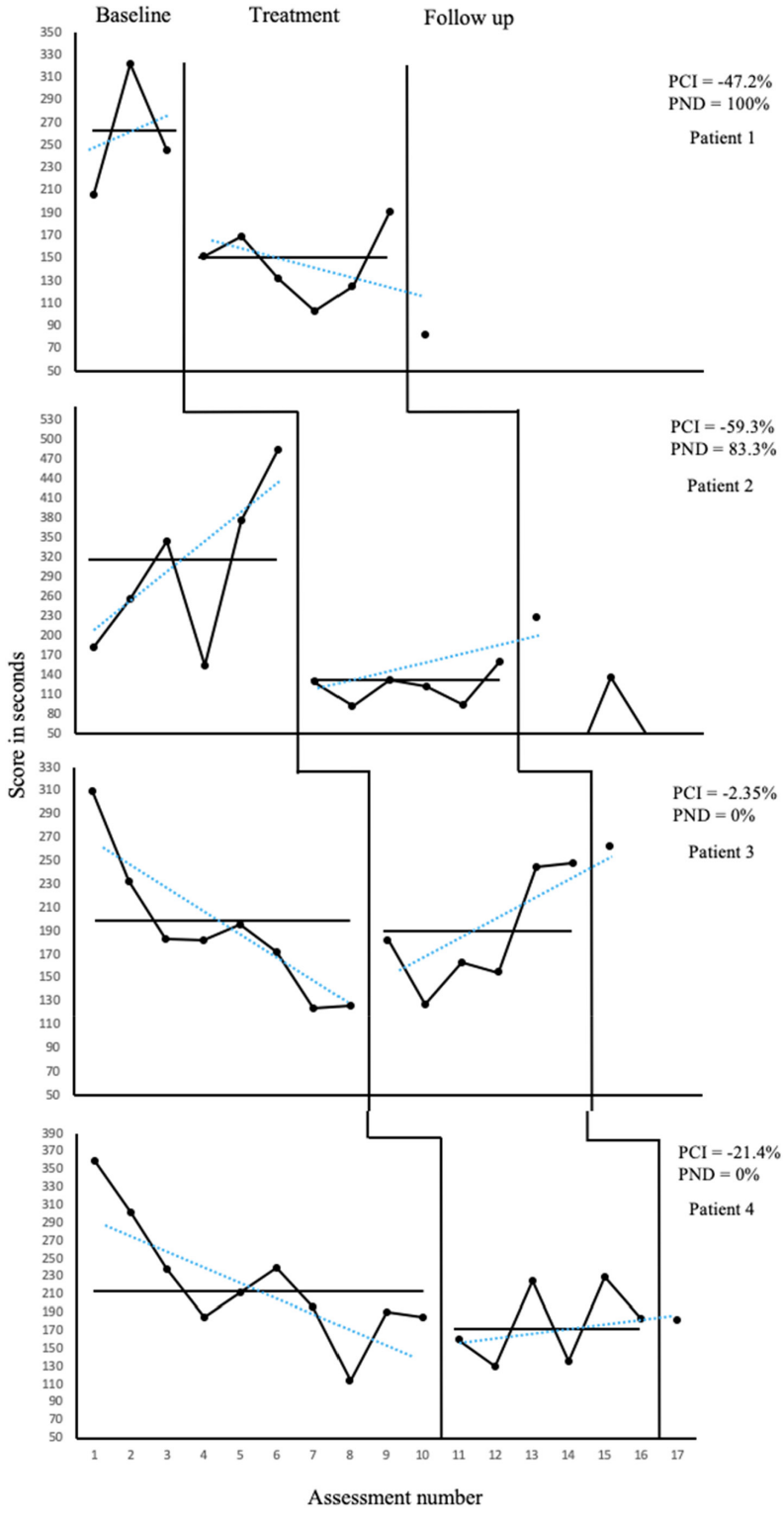

TABLE 4 Phases' characteristics for the Trail Making Test A'

Patient	Baseline condition (weeks) and total assessments	Number of assessments on the treatment phase	Baseline -Mean (SD)	Treatment - Mean (SD)
1	1 (3)	6	258.7 (59)	144.6(31.7)
2	3 (6)	6	299.7 (125.7)	121.8 (25.4)
3	4 (8)	6	190.9 (109.3)	186.8 (49.3)
4	5 (10)	6	224.5 (72.8)	177.5 (43.2)

Note: The scores are the time (in seconds) needed to complete the task.

Abbreviation: SD, standard deviation.

Neuropsychological evaluation

Patients' average scores on the two neuropsychological evaluations are presented in Table 7. Patients' performance was found to be improved three months after the intervention on the Mini Mental State Examination (improved on average by 1 point). The immediate recall of the Logical Memory test was improved on average by 1.6 words, while the delayed recall improved on average by .6 words. Patient 1, in relation to the pre-treatment assessment, remembered 4 more items, Patient 2 remembered 1 more item and Patient 4 improved their performance by 1.5 items. No difference was observed in Patient 3. The phonological verbal fluency increased from 16.75 average recalled words to 20.25. The time needed to complete the Trail Making Test A' was reduced by 51.75 s. The effect was evident on Patient 1 (pre-treatment: 132 sec, follow-up: 106 s), Patient 2 (pre-treatment: 104 sec, follow-up: 54 sec) and, Patient 4 (pre-treatment: 330 sec, follow-up: 201 sec). No change was observed on Patient 3 (pre-treatment: 252 sec, follow-up: 250 sec). A decrease was observed in patients' anxiety symptoms (drop of 3.25 points) and in their neuropsychiatric symptoms (drop of 2.75 points; Table 7).

DISCUSSION

The main purpose of this study was to investigate whether gamma TMS delivered bilaterally for 10 days to the precuneus will improve episodic memory performance in patients with mild to moderate probable AD. Specifically, we hypothesized that 40 Hz precuneus stimulation for 10 days will lead to episodic memory improvement followed by general cognitive enhancement. Our single case data, that were obtained throughout the baseline phases presented great variability, making the visual inspection and the detection of the treatment effect unclear. Stable performance in baseline is necessary in single case designs to allow for safe conclusions (Kratochwill et al., 2010). Even in the absence of stability, improved immediate recall was observed for three participants by the increased mean level and the PCI index. Additionally, PND and NAP scores indicated statistically significant treatment effect on the two patients' attention skills as evaluated by the Trail Making Test A', while a visually observed, but not statistically significant, effect was evident in Patient 4.

Furthermore, our results demonstrated an effective immediate treatment effect in all patients' ADAS-cog scores, which was maintained and improved further at 3 months post-treatment. Our neuropsychological data suggest that at 3 months post-treatment, gamma TMS intervention induced an average increase of 1.6 immediate recalled items in logical memory test, and .6 delayed recalled items. Patients' attention improvement, was also evident in the neuropsychological evaluation, as indicated by time reduction in three of the patients. Finally, gamma TMS improved patients' phonemic verbal fluency and led to anxiety and neuropsychiatric symptoms alleviation. Overall, the results of our study show a wide and long lasting positive effect of precuneus gamma stimulation on AD patients' cognitive function, while indicated its safety and tolerability.

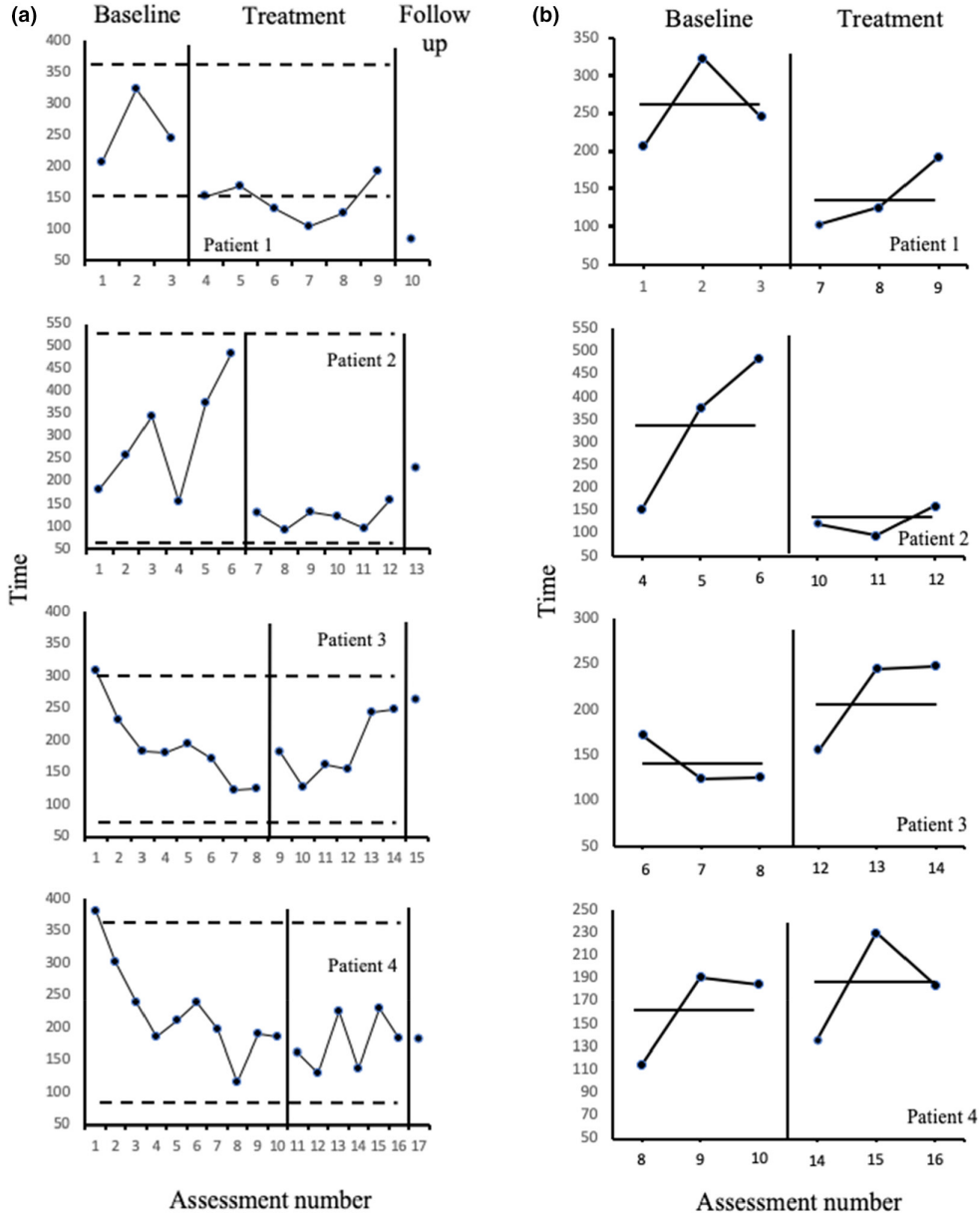

FIGURE 5 Visual analysis of the single case data of the Trail Making Test A'.
Note: (a) Two-standard deviation band around the mean. (b) Immediacy of the effect by comparing changes in mean levels of the last three data points of the baseline conditions with the last three of the treatment conditions.

A noteworthy observation is the response of Patient 3 in the TMS treatment in comparison with the other three patients. Specifically, Patient 3 presented very limited signs of improvement, in both the neuropsychological evaluations (with exception their ADAS-cog performance) and the assessments during the single case phases. As Patient 3 had similar clinical characteristics to the other three patients, a possible explanation is that the TMS protocol was applied in lower intensity in this patient (*i.e.*, 65% of the maximal machine output). It is possible that the stimulation was not strong enough to stimulate the precuneus at the same extent as for the other three patients. This observation allows to conclude

TABLE 5 Phases' characteristics for the Semantic Associations and the Naming Tasks

Patient	Baseline condition (weeks) and total assessments	Number of assessments on the treatment phase	Semantic associations		Naming	
			BM (SD)	TM (SD)	BM (SD)	TM (SD)
1	1 (3)	6	2.6 (2.3)	2 (1)	10.3 (1.2)	11.7 (.5)
2	3 (6)	6	3.5 (1.2)	3.8 (1.4)	10.6 (1.2)	11.2 (1.2)
3	4 (8)	6	1 (1.3)	1.2 (2.85)	13.5 (.75)	13.3 (1)
4	5 (10)	6	4.6 (2.3)	5 (1.4)	8.7 (1.7)	9 (1.5)

Note: The score on the semantic association task refers to the number of errors made in both the verbal and the visuospatial subtests.

Abbreviations: BM, baseline phase mean; SD, standard deviation; TM, treatment phase mean.

TABLE 6 Phases' characteristics for the Corsi Block, Forward and Backwards Tasks

Patient	Baseline condition (weeks) and total assessments	Number of assessments on the treatment phase	Corsi block task			
			Forward		Backwards	
			BM (SD)	TM (SD)	BM (SD)	TM (SD)
1	1 (3)	6	7.3 (1.2)	6 (.6)	3.3 (1.2)	4.6 (1)
2	3 (6)	6	5.8 (1.5)	6.3 (1.4)	3.3 (1.5)	4.6 (1.2)
3	4 (8)	6	3.6 (.9)	4 (.9)	3.1 (1.2)	3.3 (.8)
4	5 (10)	6	5.7 (1)	4.8 (1.9)	3.8 (.9)	2.5 (1.7)

Abbreviation: SD, standard deviation.

that, given the absence of universal golden standard protocols in the application of TMS for neurorehabilitation, even small deviations in some parameters may have significant impact to the therapy's effectiveness.

Disturbances in gamma oscillations have been observed in several neurological and psychiatric diseases, such as chronic pain (*e.g.*, Mussigmann et al., 2021) and depression (*e.g.*, Fitzgerald & Watson, 2018). Therefore, the effects of gamma frequency stimulation protocols are being investigated in both healthy (Traikapi et al., 2022) and neurological patients (Benninger et al., 2012), in an attempt to develop effective treatments. On that basis, gamma entrainment therapy in AD has gained scientific interest and recent evidence suggests that it may have the potential to reduce brain atrophy, improve gamma brain activity and functional connectivity and to improve general cognitive function in AD patients (for a review see Traikapi & Konstantinou, 2021). The effect of 40 Hz brain stimulation in AD patients has been previously investigated through sensory stimulation (*e.g.*, Chan et al., 2021; He et al., 2021) and non-invasive techniques such as transcranial alternating current stimulation (tACS; *e.g.*, Benussi et al., 2021; Kehler et al., 2020), reporting encouraging results. To our knowledge, our study is only the second one to investigate 40 Hz stimulation in AD via TMS (Liu et al., 2022) but the first to provide evidence about the safety and the cognitive effects of this specific protocol. Our results are in line with the previous studies reporting patients' improvement in the ADAS-cog (*e.g.*, Liu et al., 2022), but we have further investigated in more depth the effect on patients' cognitive function, which indicated a wider positive effect. Our results, reinforce the evidence that TMS at gamma frequency is safe and tolerable and provide further evidence and support of the view that TMS could represent a promising and effective non-pharmacological intervention for improving cognitive impairment in AD (*e.g.*, Koch et al., 2018; Liu et al., 2022; Rabey et al., 2013; Zhao et al., 2017).

While the exact mechanisms underlying TMS-induced cognitive changes were not investigated here, recent evidence suggests that gamma stimulation through TMS has the potential to increase local, long-range, and dynamic connectivity within the brain, while it can modulate gamma-band oscillations and prevent grey matter volume loss (Liu et al., 2022). Future work can investigate whether our TMS protocol, applied bilaterally to the precuneus, enhanced gamma brain activity and

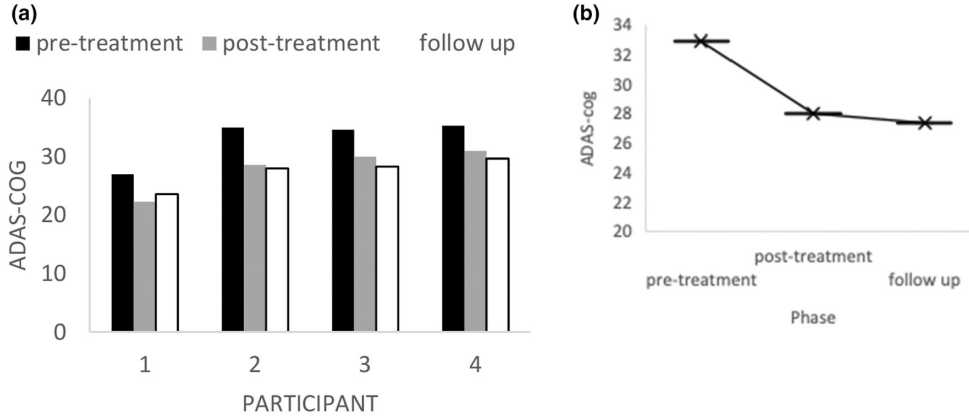

FIGURE 6 ADAS-cog scores in pre-treatment, post-treatment and follow up phases.
Note: The ADAS-cog score is based on errors made in each subtest. The highest score (*i.e.*, 80) indicates severe impairment while the least impairment is indicated by the minimum score (*i.e.*, 0). Therefore, drop in score indicates cognitive improvement. (α) Patients' ADAS-cog total scores at the three phases. (b) Differences in the average ADAS-cog scores of the four patients on each phase.

TABLE 7 Neuropsychological assessment at pre-treatment and follow-up

Cognitive domain	Neuropsychological test	Average pre-treatment score	Average follow up score
Cognitive status	Mini mental state examination	20	21
Memory	Logical memory		
	Immediate recall	2.25	3.87
	Delayed recall	0	.63
	Rey osterrieth complex figure immediate recall	3.25	4.13
	Delayed RECALL	2.37	3.5
Attention	Trail making test A'	204.5	152.75
	Digit span forward	7	7
Working memory	Digit span backwards	4.25	5
Visuospatial Abilities/Praxis	Rey osterrieth complex figure test - Copy	24	24
Executive functions	Phonological verbal fluency	16.75	20.25
	Frontal assessment battery	12.25	12.25
	Trail making B'	-	-
Mood/Psychiatric symptoms	Geriatric depression scale −15	4.75	3.75
	Beck anxiety inventory	4.75	1.5
	Neuropsychiatric inventory	10	7.25

Note: The table shows the average scores and standard deviation of patients' performance, obtained by neuropsychological testing before the study and 3 months after the two-week Transcranial Magnetic Stimulation intervention. The interval between the two neuropsychological assessments was between 5 and 6 months, depending on the patients' baseline condition. The Trail Making Test B' was not completed by any patient.

connectivity within the DMN leading to cognitive improvement. Similar findings have been reported recently by He et al. (2021), who investigated the efficacy of audiovisual 40 Hz stimulation in AD patients and indicated improved functional connectivity in the DMN. Similarly, Chan et al. (2021) reported reduced functional connectivity loss, less brain atrophy and cognitive improvement in the AD patients who received 40 Hz audiovisual stimulation for 3 months, in relation to the control group.

This study was subject to some limitations. First, the clinical diagnosis of AD was not supported using the well-known biomarkers making the diagnosis of AD in the enrolled participants uncertain. Second, while alternative forms of equal difficulty were developed for patients' assessments during the baseline and treatment phases, the exact level of difficulty was not assessed. Therefore, it is possible that patients' performance was affected by some difficulty deviations. However, the used alternative forms of ADAS-cog were standardized and their equality in difficulty had been examined and established before the study. Third, the obtained single case data failed to establish stable patients' performance during the baseline conditions, making the comparison between phases unsafe. With this possibility in mind, our study's protocol involved neuropsychological evaluations, with a relatively acceptable interval between them. Finally, neuroimaging techniques were not employed post-treatment to detect the underling neurophysiological changes caused by the TMS treatment. Therefore, there is no evidence to support brain physiological changes caused by the stimulation and hence, the extent to which the observed cognitive improvements were affected by factors such as practice effect or anxiety and/or psychiatric symptoms alleviation, cannot be determined. Despite these limitations, our findings support a positive effect of 40 Hz TMS over the precuneus on AD patients' cognitive function.

In conclusion, this study offers preliminary evidence that gamma brain stimulation, through TMS, may have the potential to alleviate cognitive function in patients with mild to moderate probable AD. Stimulating bilaterally the precuneus at 40 Hz, can improve patients' cognitive function for up to 3 months. Our findings provide evidence regarding the efficacy of TMS as an effective non-invasive technique in neurorehabilitation.

AUTHOR CONTRIBUTIONS

Artemis Traikapi: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; visualization; writing – original draft. **Ioanna Kalli:** Investigation. **Andrea Kyriakou:** Investigation. **Elena Stylianou:** Investigation. **Rafaella Tereza Symeou:** Investigation. **Akrivi Kardama:** Resources. **Yiolanda Panayiota Christou:** Resources. **Phivos Phylactou:** Investigation. **Nikos Konstantinou:** Conceptualization; funding acquisition; investigation; methodology; supervision; visualization; writing – review and editing.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

OPEN RESEARCH BADGES

This article has earned a Preregistered Research Designs badge for having a preregistered research design, available at [https://osf.io/5yhuv/].

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

ORCID

Artemis Traikapi https://orcid.org/0000-0002-2596-9569
Ioanna Kalli https://orcid.org/0000-0002-2599-9565
Andrea Kyriakou https://orcid.org/0000-0002-2140-3242
Elena Stylianou https://orcid.org/0000-0002-2158-4768
Rafaella Tereza Symeou https://orcid.org/0000-0001-9851-7902
Phivos Phylactou https://orcid.org/0000-0002-7333-8761
Nikos Konstantinou https://orcid.org/0000-0003-4531-3636

REFERENCES

Adaikkan, C., Middleton, S. J., Marco, A., Pao, P. C., Mathys, H., Kim, D. N. W., Gao, F., Young, J. Z., Suk, H. J., Boyden, E. S., McHugh, T., & Tsai, L. H. (2019). Gamma entrainment binds higher-order brain regions and offers neuroprotection. *Neuron*, *102*(5), 929–943.e8. https://doi.org/10.1016/j.neuron.2019.04.011

Ahmed, M. A., Darwish, E. S., Khedr, E. M., & Ali, A. M. (2012). Effects of low versus high frequencies of repetitive transcranial magnetic stimulation on cognitive function and cortical excitability in Alzheimer's dementia. *Journal of Neurology*, *259*, 83–92. https://doi.org/10.1007/s00415-011-6128-4

Bäckman, L., Small, B. J., & Fratiglioni, L. (2001). Stability of the preclinical episodic memory deficit in Alzheimer's disease. *Brain*, *124*(1), 96–102. https://doi.org/10.1093/brain/124.1.96

Beck, I. R., Gagneux-Zurbriggen, A., Berres, M., Taylor, K. I., & Monsch, A. U. (2012). Comparison of verbal episodic memory measures: Consortium to establish a registry for Alzheimer's disease—Neuropsychological Assessment Battery (CERAD-NAB) versus California Verbal Learning Test (CVLT). *Archives of Clinical Neuropsychology*, *27*(5), 510–519. https://doi.org/10.1093/arclin/acs056

Benninger, D. H., Iseki, K., Kranick, S., Luckenbaugh, D. A., Houdayer, E., & Hallett, M. (2012). Controlled study of 50-Hz repetitive transcranial magnetic stimulation for the treatment of parkinson disease. *Neurorehabilitation and Neural Repair*, *26*(9), 1096–1105. https://doi.org/10.1177/1545968312445636

Benussi, A., Cantoni, V., Cotelli, M. S., Cotelli, M., Brattini, C., Datta, A., Thomas, C., Santarnecchi, E., Pascual-Leone, A., & Borroni, B. (2021). Exposure to gamma tACS in Alzheimer's disease: A randomized, double- blind, sham-controlled, crossover, pilot study. *Brain Stimulation.*, *14*, 531–540. https://doi.org/10.1016/j.brs.2021.03.007

Bonnì, S., Veniero, D., Mastropasqua, C., Ponzo, V., Caltagirone, C., Bozzali, M., & Koch, G. (2015). TMS evidence for a selective role of the precuneus in source memory retrieval. *Behavioural Brain Research*, *282*, 70–75. https://doi.org/10.1016/j.bbr.2014.12.032

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H., & Del Tredici, K. (2006). Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathologica*, *112*(4), 389–404. https://doi.org/10.1007/s00401-006-0127-z

Braak, H., Braak, E., & Bohl, J. (1993). Staging of Alzheimer-related cortical destruction. *European Neurology*, *33*(6), 403-408. 389-404–403-408. 389-408. https://doi.org/10.1159/000116984

Buckner, R. L., Andrews-Hanna, J. R., & Schacter, D. L. (2008). The brain's default network: Anatomy, function, and relevance to disease. *Annals of the New York Academy of Science*, *1124*, 1–38. https://doi.org/10.1196/annals.1440.011

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

Caputi, N., Di Giacomo, D., Aloisio, F., & Passafiume, D. (2016). Deterioration of semantic associative relationships in mild cognitive impairment and Alzheimer Disease. *Applied Neuropsychology: Adult*, *23*(3), 186–195. https://doi.org/10.1080/23279095.2015.1030020

Chan, D., Suk, H. J., Jackson, B., Milman, N. P., Stark, D., Klerman, E. B., Kitchener, E., Avalos, V. S. F., Banerjee, A., Beach, S. D., Blanchard, J., Stearns, C., Boes, A., Uitermarkt, B., Gander, P., Howard III, M., Sternberg, E. J., Nieto-Castanon, A., Anteraper, S., … Tsai, L. H. (2021). Gamma frequency sensory stimulation in probable mild Alzheimer's dementia patients: Results of a preliminary clinical trial. *MedRxiv [preprint]*. https://doi.org/10.1101/2021.03.01.21252717. May 17, 2021 (Accessed 15th March 2022).

Chen, Y., Liu, Z., Zhang, J., Chen, K., Yao, L., Li, X., Gong, G., Wang, J., & Zhang, Z. (2017). Precuneus degeneration in nondemented elderly individuals with APOE □4: Evidence from structural and functional MRI analyses. *Human Brain Mapping*, *38*(1), 271–282. https://doi.org/10.1002/hbm.23359

Cotelli, M., Calabria, M., Manenti, R., Rosini, S., Zanetti, O., Cappa, S. F., & Miniussi, C. (2011). Improved language performance in Alzheimer disease following brain stimulation. *Journal of Neurology, Neurosurgery & Psychiatry*, *82*(7), 794–797. https://doi.org/10.1136/jnnp.2009.197848

Fan, L., Mao, C., Hu, X., Zhang, S., Yang, Z., Hu, Z., Sun, H., Fan, Y., Dong, Y., Yang, J., Shi, C., & Xu, Y. (2020). New insights into the pathogenesis of Alzheimer's disease. *Frontiers in Neurology*, *10*(1312). https://doi.org/10.3389/fneur.2019.01312

Fitzgerald, P. J., & Watson, B. O. (2018). Gamma oscillations as a biomarker for major depression: An emerging topic. *Translational Psychiatry*, *8*(1), 1–7. https://doi.org/10.1038/s41398-018-0239-y

Fransson, P., & Marrelec, G. (2008). The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: Evidence from a partial correlation network analysis. *NeuroImage*, *42*(2), 1178–1184. https://doi.org/10.1016/j.neuroimage.2008.05.059

Gallagher, M., & Koh, M. T. (2011). Episodic memory on the path to Alzheimer's disease. *Current Opinion in Neurobiology*, *21*(6), 929–934. https://doi.org/10.1016/j.conb.2011.10.021

Gilboa, A., Winocur, G., Grady, C. L., Hevenor, S. J., & Moscovitch, M. (2004). Remembering our past: Functional neuroanatomy of recollection of recent and very remote personal events. *Cerebral Cortex*, *14*(11), 1214–1225. https://doi.org/10.1093/cercor/bhh082

Goutagny, R., Gu, N., Cavanagh, C., Jackson, J., Chabot, J. G., Quirion, R., Krantic, S., & Williams, S. (2013). Alterations in hippocampal network oscillations and theta–gamma coupling arise before Aβ overproduction in a mouse model of Alzheimer's disease. *European Journal of Neuroscience*, *37*(12), 1896–1902. https://doi.org/10.1111/ejn.12233

Hartmann, D. P., Barrios, B. A., & Wood, D. D. (2004). Principles of behavioral observation. In S. H. Haynes (Ed.), *Comprehensive handbook of psychological assessment. Behavioral assessment* (Vol. 3, pp. 108–127). Wiley.

Hayes, S. C., Barlow, D. H., & Nelson-Gray, R. O. (1999). *The scientist practitioner: Research and accountability in the age of managed care* (2nd ed.). Allyn & Bacon.

He, Q., Colon-Motas, K. M., Pybus, A. F., Piendel, L., Seppa, J. K., Walker, M. L., Manzanares, C. M., Qiu, D., Miocinovic, S., Wood, L. B., Levey, A. I., Lah, J. J., & Singer, A. C. (2021). A feasibility trial of gamma sensory flicker for patients with prodromal Alzheimer's disease. *Alzheimer's & Dementia*, *7*, 1–11. https://doi.org/10.1002/trc2.12178

Hebscher, M., Ibrahim, C., & Gilboa, A. (2020). Precuneus stimulation alters the neural dynamics of autobiographical memory retrieval. *NeuroImage*, *210*, 116575. https://doi.org/10.1016/j.neuroimage.2020.116575

Iaccarino, H. F., Singer, A. C., Martorell, A. J., Rudenko, A., Gao, F., Gillingham, T. Z., Mathys, H., Seo, J., Kritskiy, O., Abdurrob, F., Adaikkan, C., Canter, R. G., Rueda, R., Brown, E. N., Boyden, E. S., & Tsai, L. H. (2016). Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature*, *540*(7632), 230–235. https://doi.org/10.1038/nature20587

Kehler, L., Francisco, C. O., Uehara, M. A., & Moussavi, Z. (2020). The effect of transcranial alternating current stimulation (tACS) on cognitive function in older adults with dementia. *IEEE Engineering in Medicine and Biology Society Annual International Conference*, *2020*, 3649–3653. https://doi.org/10.1109/EMBC44109.2020.9175903

Kim, J., Kim, Y. H., & Lee, J. H. (2013). Hippocampus–precuneus functional connectivity as an early sign of Alzheimer's disease: A preliminary study using structural and functional magnetic resonance imaging data. *Brain Research*, *1945*, 18–29. https://doi.org/10.1016/j.brainres.2012.12.011

Koch, G., Bonnì, S., Pellicciari, M. C., Casula, E. P., Mancini, M., Esposito, R., Ponzo, V., Picazio, S., Di Lorenzo, F., Serra, L., Motta, C., Maiella, M., Marra, C., Cercignani, M., Martorana, A., Caltagirone, C., & Bozzali, M. (2018). Transcranial magnetic stimulation of the precuneus enhances memory and neural activity in prodromal Alzheimer's disease. *NeuroImage*, *169*, 302–311. https://doi.org/10.1016/j.neuroimage.2017.12.048

Krasny-Pacini, A., & Evans, J. (2018). Single-case experimental designs to assess intervention effectiveness in rehabilitation: A practical guide. *Annals of Physical and Rehabilitation Medicine*, *61*(3), 164–179. https://doi.org/10.1016/j.rehab.2017.12.002

Kratochwill, T. R., Hitchcock, J., Horner, R. H., Levin, J. R., Odom, S. L., Rindskopf, D. M., & Shadish, W. R. (2010). Single-case designs technical documentation. https://eric.ed.gov/?id=ED510743

Kratochwill, T. R., & Levin, J. R. (2014). Enhancing the scientific credibility of single-case intervention research: Randomization to the rescue. *Psychological Methods*, *15*, 124–144. https://doi.org/10.1037/a0017736

Krause, B. J., Schmidt, D., Mottaghy, F. M., Taylor, J., Halsband, U., Herzog, H., Tellmann, L., & Müller-Gärtner, H. W. (1999). Episodic retrieval activates the precuneus irrespective of the imagery content of word pair associates: A PET study. *Brain*, *122*(2), 255–263. https://doi.org/10.1093/brain/122.2.255

Kwok, S. C., Shallice, T., & Macaluso, E. (2012). Functional anatomy of temporal organisation and domain-specificity of episodic memory retrieval. *Neuropsychologia*, *50*(12), 2943–2955. doi:10.1016/j.neuropsychologia.2012.07.025

Liu, C., Han, T., Xu, Z., Liu, J., Zhang, M., Du, J., Zhou, Q., Duan, Y., Li, Y., Wang, J., Cui, D., & Wang, Y. (2022). Modulating gamma oscillations promotes brain connectivity to improve cognitive impairment. *Cerebral Cortex.*, *32*(12), 2644–2656. https://doi.org/10.1093/cercor/bhab371

Lobo, M. A., Moeyaert, M., Cunha, A. B., & Babik, I. (2017). Single-case design, analysis, and quality assessment for intervention research. *Journal of Neurologic Physical Therapy: JNPT*, *41*(3), 187–197. https://doi.org/10.1097/npt.0000000000000187

Lundstrom, B. N., Ingvar, M., & Petersson, K. M. (2005). The role of precuneus and left inferior frontal cortex during source memory episodic retrieval. *NeuroImage*, *27*(4), 824–834. https://doi.org/10.1016/j.neuroimage.2005.05.008

Mancini, M., Mastropasqua, C., Bonnì, S., Ponzo, V., Cercignani, M., Conforto, S., Koch, G., & Bozzali, M. (2017). Theta burst stimulation of the precuneus modulates resting state connectivity in the left temporal pole. *Brain Topography*, *30*(3), 312–319. https://doi.org/10.1007/s10548-017-0559-x

Martorell, A. J., Paulson, A. L., Suk, H. J., Abdurrob, F., Drummond, G. T., Guan, W., Young, J. Z., Kim, D. N. W., Kritskiy, O., Barker, S. J., Mangena, V., Prince, S. M., Brown, E. N., Chung, K., Boyden, E. S., Singer, A. C., & Tsai, L. H. (2019). Multi-sensory gamma stimulation ameliorates Alzheimer's-associated pathology and improves cognition. *Cell*, *177*(2), 256–271. https://doi.org/10.1016/j.cell.2019.02.014

Mattsson, N., Insel, P. S., Donohue, M., Jögi, J., Ossenkoppele, R., Olsson, T., Scholl, M., Smith, R., & Hansson, O. (2019). Predicting diagnosis and cognition with 18F-AV-1451 tau PET and structural MRI in Alzheimer's disease. *Alzheimer's & Dementia*, *15*(4), 570–580. https://doi.org/10.1016/j.jalz.2018.12.001

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack Jr, C. R., Kawas, C. H., Klunk, W. E., Koroshetz, W. J., Manly, J. J., Mayeux, R., Mohs, R. C., Morris, J. C., Rossor, M. N., Scheltens, P., Carrillo, M. C., Thies, B., Weintraub, S., & Phelps, C. H. (2011). The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia*, *7*(3), 263–269. https://doi.org/10.1016/j.jalz.2011.03.005

Mussigmann, T., Lefaucheur, J. P., & McGonigal, A. (2021). Gamma-band activities in the context of pain: A signal from brain or muscle? *Neurophysiologie Clinique*, *51*, 287–289. https://doi.org/10.1016/j.neucli.2021.03.007

Nestor, P. J., Scheltens, P., & Hodges, J. R. (2004). Advances in the early detection of Alzheimer's disease. *Nature Medicine*, *10*(7), S34–S41. https://doi.org/10.1038/nrn1433

Perdices, M., & Tate, R. L. (2009). Single-subject designs as a tool for evidence-based clinical practice: Are they unrecognised and undervalued? *Neuropsychological Rehabilitation*, *19*(6), 904–927. https://doi.org/10.1002/jcad.12038

Petersen, R. C., Smith, G. E., Ivnik, R. J., Kokmen, E., & Tangalos, E. G. (1994). Memory function in very early Alzheimer's disease. *Neurology*, *44*(5), 867. https://doi.org/10.1212/wnl.44.5.867

Price, J. L., Davis, P. B., Morris, J. C., & White, D. L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiology of Aging*, *12*(4), 295–312. https://doi.org/10.1016/0197-4580(91)90006-6

Rabey, J. M., Dobronevsky, E., Aichenbaum, S., Gonen, O., Marton, R. G., & Khaigrekht, M. (2013). Repetitive transcranial magnetic stimulation combined with cognitive training is a safe and effective modality for the treatment of Alzheimer's disease: A randomized, double-blind study. *Journal of Neural Transmission*, *120*(5), 813–819. https://doi.org/10.1007/s00702-012-0902-z

Reitan, R. M. (1955). The relation of the trail making test to organic brain damage. *Journal of Consulting Psychology*, *19*(5), 393–398. https://doi.org/10.1037/h0044509

Rose, N. S., LaRocque, J. J., Riggall, A. C., Gosseries, O., Starrett, M. J., Meyering, E. E., & Postle, B. R. (2016). Reactivation of latent working memories with transcranial magnetic stimulation. *Science*, *354*(6316), 1136–1139. https://doi.org/10.1126/science.aah7011

Rugg, M. D., Otten, L. J., & Henson, R. N. (2002). The neural basis of episodic memory: Evidence from functional neuroimaging. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *1424*(357), 1097–1110. https://doi.org/10.1098/rstb.2002.1102

Schmidt, D., Krause, B. J., Mottaghy, F. M., Halsband, U., Herzog, H., Tellmann, L., & Müller-Gärtner, H. W. (2002). Brain systems engaged in encoding and retrieval of word-pair associates independent of their imagery content or presentation modalities. *Neuropsychologia*, *40*(4), 457–470. https://doi.org/10.1016/s0028-3932(01)00102-6

Schrag, A., Schott, J. M., & Initiative, A.'s. D. N. (2012). What is the clinically relevant change on the ADAS-Cog? *Journal of Neurology, Neurosurgery, and Psychiatry*, *83*(2), 171–173. https://doi.org/10.1136/jnnp-2011-300881

Scruggs, T. E., & Mastropieri, M. A. (1998). Summarizing single-subject research: Issues and applications. *Behavior Modification*, *22*(3), 221–242. https://doi.org/10.1177/01454455980223001

Shallice, T., Fletcher, P., Frith, C. D., Grasby, P., Frackowiak, R. S. J., & Dolan, R. J. (1994). Brain regions associated with acquisition and retrieval of verbal episodic memory. *Nature*, *6472*(368), 633–635. https://doi.org/10.1038/368633a0

Sorg, C., Riedl, V., Mühlau, M., Calhoun, V. D., Eichele, T., Läer, L., Drzezga, A., Forstl, H., Kurz, A., Zimmer, C., & Wohlschläger, A. M. (2007). Selective changes of resting-state networks in individuals at risk for Alzheimer's disease. *Proceedings of the National Academy of Sciences*, *104*(47), 18760–18765. https://doi.org/10.1073/pnas.0708803104

Traikapi, A., & Konstantinou, N. (2021). Gamma oscillations in Alzheimer's disease and their potential therapeutic role. *Frontiers in Systems Neuroscience*, *15*. https://doi.org/10.3389/fnsys.2021.782399

Traikapi, A., Phylactou, P., & Konstantinou, N. (2022). Repetitive magnetic stimulation of the human motor cortex in the gamma band reduces cortical excitability. *Neurophysiologie Clinique*, *52*(5), 407–409. https://doi.org/10.1016/j.neucli.2022.09.005

Uhlhaas, P. J., & Singer, W. (2006). Neural synchrony in brain disorders: Relevance for cognitive dysfunctions and pathophysiology. *Neuron*, *52*(1), 155–168. https://doi.org/10.1016/j.neuron.2006.09.020

Vannest, K. J., & Ninci, J. (2015). Evaluating intervention effects in single-case research designs. *Journal of Counseling & Development*, *93*(4), 403–411. https://doi.org/10.1002/jcad.12038

Vannest, K. J., Parker, R. I., Gonen, O., & Adiguzel, T. (2016). *Single case research: Web based calculators for SCR analysis . (Version 2.0) [Web-based application]*. Texas A&M University singlecaseresearch.org [Accessed 27th Tuesday September 2022].

Ye, Q., Zou, F., Dayan, M., Lau, H., Hu, Y., & Kwok, S. C. (2019). Individual susceptibility to TMS affirms the precuneal role in meta-memory upon recollection. *Brain Structure and Function*, *224*(7), 2407–2419. https://doi.org/10.1007/s00429-019-01909-6

Yue, W. U., Wenwei, X. U., Xiaowei, L. I. U., Qing, X. U., Li, T. A. N. G., & Shuyan, W. U. (2015). Adjunctive treatment with high frequency repetitive transcranial magnetic stimulation for the behavioral and psychological symptoms of patients with Alzheimer's disease: A randomized, double-blind, sham-controlled study. *Shanghai Archives of Psychiatry*, *27*(5), 280–288. https://doi.org/10.11919/j.issn.1002-0829.215107

Zhao, J., Li, Z., Cong, Y., Zhang, J., Tan, M., Zhang, H., Geng, N., Li, M., Yu, W., & Shan, P. (2017). Repetitive transcranial magnetic stimulation improves cognitive function of Alzheimer's disease patients. *Oncotarget*, *8*(20), 33864–33871. https://doi.org/10.18632/oncotarget.13060

Zhao, Q., Lv, Y., Zhou, Y., Hong, Z., & Guo, Q. (2012). Short-term delayed recall of auditory verbal learning test is equivalent to long-term delayed recall for identifying amnestic mild cognitive impairment. *PLoS One*, *7*(12), e51157. https://doi.org/10.1371/journal.pone.0051157

Zhou, B., Yao, H., Wang, P., Zhang, Z., Zhan, Y., Ma, J., Xu, K., Wang, L., An, N., Liu, Y., & Zhang, X. (2015). Aberrant functional connectivity architecture in Alzheimer's disease and mild cognitive impairment: A whole-brain, data-driven analysis. *BioMed Research International*, *2015*. https://doi.org/10.1155/2015/495375

How to cite this article: Traikapi, A., Kalli, I., Kyriakou, A., Stylianou, E., Symeou, R. T., Kardama, A., Christou, Y. P., Phylactou, P., & Konstantinou, N. (2022). Episodic memory effects of gamma frequency precuneus transcranial magnetic stimulation in Alzheimer's disease: A randomized multiple baseline study. *Journal of Neuropsychology*, *00*, 1–23. https://doi.org/10.1111/jnp.12299