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Ultrasound Neuromodulation With Transcranial Pulse Stimulation in Alzheimer Disease

A Randomized Clinical Trial

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Abstract

IMPORTANCE Given the increasing prevalence of dementia and the limited treatment options available, ultrasound neuromodulation could serve as a novel add-on therapy to standard treatments for Alzheimer disease (AD). As ultrasound neuromodulation is still in its early stages, further research is essential to fully explore its potential in treating brain disorders.

OBJECTIVE To evaluate clinical and functional imaging effects of transcranial pulse stimulation (TPS) in patients with AD.

DESIGN, SETTING, AND PARTICIPANTS A randomized, double-blind, sham-controlled, crossover clinical trial was conducted at the Medical University of Vienna between January 1, 2017, and July 27, 2022. Sixty patients with clinically diagnosed AD receiving state-of-the-art treatment were randomly allocated to treatment sequence groups verum-sham (first cycle verum, second cycle sham, n = 30) and sham-verum (n = 30). Data analysis was performed from July 28, 2022, to September 5, 2024.

INTERVENTION Each participant received 6 verum and 6 sham TPS sessions (6000 pulses, 0.20 mJ/mm^2, 5 Hz) to frontoparietal brain areas.

MAIN OUTCOMES AND MEASURES Neuropsychological tests, including the primary outcome Consortium to Establish a Registry for Alzheimer's Disease (CERAD) corrected total score (CTS), were performed at baseline and 1 week, 1 month, and 3 months following the stimulations in each cycle. Primary and secondary outcomes, including functional magnetic resonance imaging and Beck Depression Inventory-II, were analyzed by intention-to-treat analysis and, for sensitivity, by per protocol analysis.

RESULTS For the intention-to-treat analysis, 60 patients between ages 51 and 82 years (mean [SD], 70.65 [8.16] years; 30 females; 30 males) were included. The CERAD CTS increased by a mean (SD) of 2.22 (6.87) points in the verum condition from 70.93 (14.27) points at baseline to 73.15 (14.90) 3 months after stimulation, while the mean (SD) score in the sham condition increased by 1.00 (6.82) point vs baseline from 71.68 (13.62] at baseline to 72.68 (14.48) 3 months after stimulation. Primary data analysis of the condition × session interaction was not significant (P = .68; partial η^2 [ηp^2] = 0.01), but its interaction with age was P = .003; ηp^2 = 0.08, followed by post hoc analyses of age subsamples. Although several patients older than 70 years benefited from verum TPS, only the younger subgroup (≤70 years) showed significantly higher CTS increases for verum in all poststimulation sessions (condition × session: P = .005; ηp^2 = 0.16). At 3 months after stimulation, for example, a mean (SD) 3.91 (7.86)-point increase was found for verum TPS in the younger patients,

(continued)

Key Points

Question Does ultrasound neuromodulation using transcranial pulse stimulation (TPS) improve cognitive functions in patients with Alzheimer disease (AD)?

Findings In a randomized clinical trial in 60 patients with AD receiving state-of-the-art therapy, 2 weeks of verum TPS significantly improved cognitive scores in a younger subsample of patients with AD. In addition, TPS upregulated memory-associated brain activation and functional connectivity in the attention network.

Meaning In this study, transcranial pulse stimulation was well tolerated and enhanced cognition and related brain networks, suggesting the potential utility of TPS as a novel add-on therapy in AD.

+ Supplemental content

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Abstract (continued)

but a mean (SD) CTS decrease of 1.83 (5.80) was observed for sham. Memory-associated brain activation was significantly higher after verum TPS in the precuneus, visual, and frontal areas, while resting state functional connectivity was significantly upregulated in the dorsal attention network. In the per protocol sample, a significant reduction of the Beck Depression Inventory-II scores 3 months following verum TPS was found (verum baseline: 7.27 [5.87]; verum 3 months after stimulation: 5.27 [5.27]; sham baseline: 6.70 [5.65]; sham 3 months after stimulation: 6.22 [4.40]; $P = .008$; $\eta p^2 = 0.23$). During both verum and sham conditions, the most common observed adverse symptom was depression; no major neuropathologic change was detected in the patients by detailed neuroradiologic assessments.

CONCLUSIONS AND RELEVANCE In this randomized clinical trial of TPS in patients with AD, a 2-week verum treatment improved cognitive scores in the younger subgroup, ameliorated depressive symptoms, and induced upregulation of functional brain activation and connectivity. These findings suggest TPS may be a safe and promising add-on therapy for patients with AD receiving state-of-the-art treatment.

TRIAL REGISTRATION ClinicalTrials.gov Identifier: NCT03770182

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Introduction

Alzheimer disease (AD), the most common form of dementia, is neuropathologically characterized by the progressive accumulation of amyloid β plaques and tau tangles. Clinically, AD follows a continuum that begins with a presymptomatic phase, advances to mild cognitive impairment, and eventually progresses to dementia.[1] Most pharmacologic treatments, including novel monoclonal antibodies, are approved for early stages only, and their clinical effects and risk-benefit ratio remain under debate.[2-4] Noninvasive brain stimulation has demonstrated the potential to further enhance clinical outcomes for patients already receiving state-of-the-art therapy.[5,6] In particular, the pioneering development of focused navigated ultrasound neuromodulation has attracted increasing attention.[7-10] In contrast to electric (eg, transcranial direct current stimulation) or magnetic techniques (eg, transcranial magnetic stimulation), ultrasound neuromodulation exhibits specific advantages for therapeutic applications: (1) brain abnormalities may not considerably mislead the stimulation focus as possible with electromagnetic field distortions,[11] (2) noninvasive modulation of deep brain areas is possible,[12] and (3) the stimulation is highly focal.[13]

In an uncontrolled setting, the first study[14] with navigated focused ultrasound neuromodulation in AD indicated potential cognitive, functional magnetic resonance imaging (fMRI), and morphologic effects in patients already receiving standard treatments.[14-17] Combining clinical data with multimodal MRI, we provide what is, to our knowledge, the first sham-controlled randomized clinical trial on focused ultrasound neuromodulation in AD to evaluate the clinical potential of this novel add-on therapy.

Methods

Study Design

This randomized, double-blind, sham-controlled clinical trial was conducted at the Medical University of Vienna and obtained the ethical approval from the ethics committee of the Medical University of Vienna. This study adhered to the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline for randomized clinical trials. All participants were receiving stable state-of-the art

treatment and signed written informed consent forms; participants received financial compensation.

General TPS methods and evaluation settings closely followed the pilot study.[14] Participants received verum and sham TPS stimulation in a crossover design and were randomly allocated to the intervention sequence groups verum-sham (first cycle verum, second cycle sham) and sham-verum (eMethods in Supplement 2). Both cycles included baseline assessments, 6 TPS interventions within 2 weeks, and follow-ups 1 week, 1 month, and 3 months after completing the stimulations (eFigure 1 in Supplement 2). The washout period between both cycles lasted 5 weeks, leading to an interval of approximately 4 months between verum and sham interventions.

Participants

The study population comprised 60 patients whose condition was clinically stable with mild cognitive impairment or probable AD according to clinical National Institute on Aging and Alzheimer's Association (NIA-AA) criteria[18] or *International Statistical Classification of Diseases and Related Health Problems, 10th Revision* (*ICD-10*) criteria (code F00.). Participants were recruited via media reports and advertisements and from the Department of Neurology, Medical University of Vienna. Exclusion criteria were nonadherence to the protocol, relevant intracerebral pathologic factors unrelated to AD, hemophilia or other blood clotting disorders, thrombosis, corticosteroid treatment within the last 6 weeks, and women who were pregnant or breastfeeding.

Procedures

Participants received 6 TPS applications over 2 weeks per cycle (6 verum plus 6 sham TPS applications in total), using a modified Duolith SD1 (Storz Medical AG). This system was the prototype of the Neurolith,[14] which received the Conformité Européene mark of the European Union for AD treatment in 2018. Per session, 6000 TPS pulses were applied, with 0.20-mJ/mm^2 energy flux density, 3-µs pulse duration, 5-Hz pulse repetition frequency, duty cycle = 0.000015, and a multifrequency band predominantly between 0.05 and 0.45 MHz. Considering a skull attenuation of 80% to 90%,[14] typical maximum pulse characteristics below the skull were 24 mW/cm^2 spatial peak temporal average intensity, 1.6 kW/cm^2 spatial peak pulse average intensity, and ambient pressure–related positive (7.5 MPa) and negative (−6 MPa) peak pressures. Brain stimulation targets comprised bilateral dorsolateral prefrontal cortex and inferior frontal cortex, bilateral lateral parietal cortex extending to the Wernicke area, and precuneus cortex (eFigure 2 in Supplement 2). For sham stimulation, a cap on the TPS hand piece blocking the ultrasound waves was applied, looking and sounding identical to the verum cap (eMethods in Supplement 2).

Neuropsychological assessments were administered in the first week of each cycle (baseline), after the treatments in week 5 (post stimulation), week 8 (1 month post stimulation), and week 16 (3 months post stimulation), maintaining the same structure across both study cycles (eMethods in Supplement 1). For both cycles, MRI measurements were recorded the week before and the week after TPS stimulations, resulting in 4 MRI sessions in total. The MRI measurements were performed using a 3-T instrument with a 64-channel head coil (Prisma MR; Siemens). A T1-weighted structural image, T2-weighted fast low-angle shot and fluid-attenuated inversion recovery sequences to evaluate potential neuropathologic characteristics, and T2*-weighted EPI data for the memory task (eFigure 3 in Supplement 2) and resting state fMRI were recorded (eMethods in Supplement 2).

Outcomes

As a reliable measure for cognitive deficits due to dementia, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) corrected total score (CTS) constituted the primary outcome for this study.[19,20] Secondary outcomes included the cognitive test batteries (Neuropsychological Test Battery Vienna and Alzheimer Disease Assessment Scale [ADAS], ADAS-Sum, ADAS-Cog, and clock drawing test), depression inventories (Beck Depression Inventory-II [BDI-II], Geriatric Depression Scale), self and external assessments of forgetfulness (Forgetfulness Assessment Inventory),

activities of daily living (Instrumental Activities of Daily Living Scale, Bayer Activities of Daily Living Scale), leisure behavior, the Prosopagnosia Index, and the Neuropsychiatric Inventory. Functional imaging outcomes comprised memory task fMRI activation and functional connectivity as assessed by the global efficiency (GE) within brain networks during resting-state fMRI.

Adverse events were systematically collected through interviews with patients and accompanying persons. The eMethods in Supplement 2 includes further details regarding study outcomes.

Statistical Analysis

The sample size calculation was based on CERAD CTS improvement in pilot data, resulting in a minimal sample size of 24 patients per group. To account for dropouts, 30 patients were included per treatment sequence group.

For initial focused outcome analysis, behavioral variables were independently analyzed by a contract research organization, testing the difference between treatment sequence groups regarding score changes using *t* tests (eMethods in Supplement 2). This was complemented by a repeated-measures analysis of variance (rmANOVA) as predefined in the clinical investigation plan and necessary to evaluate main effects and interactions.[14] Analysis of neuropsychological outcomes was conducted for the intention-to-treat (ITT) sample, including dropouts with missing values imputed using last-observation-carried-forward, and for the per-protocol (PP) sample as secondary supporting analysis. Partial η^2 (ηp^2) was used to measure effect size, with thresholds of 0.01 indicating a small effect, 0.06 a medium effect, and 0.14 suggesting a large effect.

Behavioral data analysis was performed using SPSS Statistics, version 28.0 (IBM Corp). An rmANOVA with the within-patient factors condition (verum and sham) and session (baseline, post stimulation, 1-month post stimulation, 3-month post stimulation) and the interaction condition × session was conducted for the primary outcome variable CERAD CTS and behavioral secondary outcomes. The level of significance was set at $P < .05$ (2-sided). Significant interactions between condition and session were followed by post hoc comparisons of treatment effect (verum and sham) for each post stimulation time point vs baseline, adjusted for multiple comparisons using Bonferroni-Holm correction.

Differences between both groups regarding potential biasing baseline characteristics were addressed by entering the confounding variable as a covariate in a post hoc rmANCOVA. Pearson correlation analysis between age and the CERAD CTS outcome was conducted. To rule out possible influences of carryover effects, a post hoc parallel-group analysis of the first cycle was performed by calculating an rmANCOVA with group as a between-patient factor, session as a within-patient factor, and relevant baseline characteristics as covariates.

The fMRI memory task data were analyzed using SPM12, version 7771 (Wellcome Trust Centre for Neuroimaging). After preprocessing and patient-level statistics of novel vs repeated face-name associations, a flexible factorial design with the within-patient factors condition (verum and sham) and session (baseline and post stimulation), as well as the factor patient, was applied on group level.

Analysis of resting-state fMRI data was performed using the CONN toolbox, version 22a (Computational Neuroscience Research Lab).[21] Following preprocessing and denoising, functional connectivity matrices were calculated for every patient and group statistics were entered using the graph theoretical measure GE within several cognitive networks.

Data analysis was performed from July 28, 2022, to September 5, 2024. Further details regarding the fMRI task and functional connectivity analysis are available in the eMethods in Supplement 2.

Results

Study Population

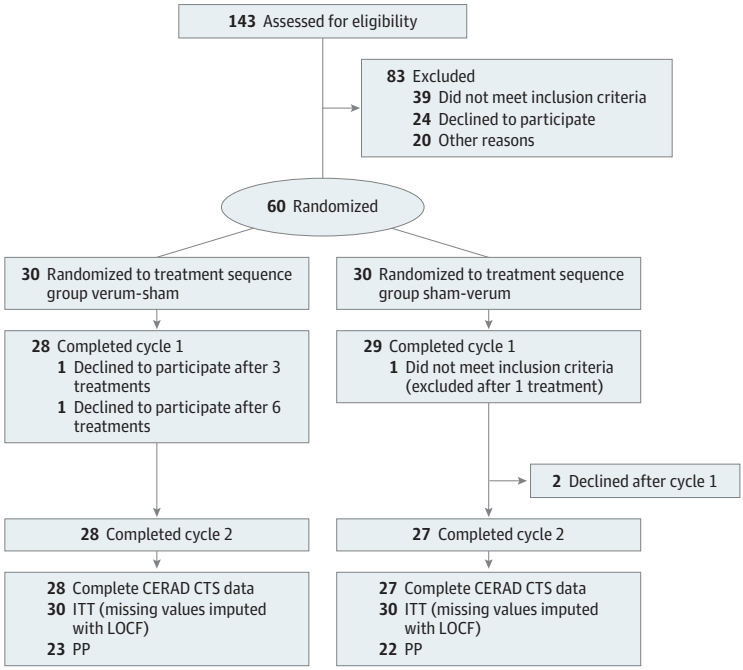
Between January 1, 2017, and July 27, 2022, 143 individuals were recruited, and after comprehensive screening for eligibility, 60 were randomized to the groups verum-sham and sham-verum (30 each)

(**Figure 1**). According to NIA-AA criteria,[18] 31 patients (52%) were diagnosed with probable AD and 14 (23%) with mild cognitive impairment due to AD. In addition, 15 patients were diagnosed based on *ICD-10* as meeting the criteria for either dementia in AD (13 [22%]) or mild cognitive impairment (2 [3%]). The NIA-AA criteria exclude substantial concomitant cerebrovascular disease, which was also assessed in patients whose condition was diagnosed according to *ICD-10* using the Fazekas score. Only 1 patient (verum-sham group) showed a Fazekas score of 3 during the eligibility screening, indicating vascular comorbidity in this individual. There were no statistically significant differences regarding baseline cognitive abilities as assessed by the Mini-Mental State Examination (MMSE) between patients with a diagnosis according to NIA-AA (n = 45; mean [SD], 23.91 [3.22]) and *ICD-10* (n = 15; mean [SD], 23.22 [2.99]) (P = .54; unpaired t test, 2-sided).

For 5 patients, study participation terminated ahead of time. A small, preexisting subarachnoid hemorrhage was detected in 1 participant during the baseline MRI, resulting in exclusion from the study. Another patient developed a mild transient ischemic attack after completing the first cycle (sham). Three participants withdrew from the study due to the burden of the study appointments: one after 3 verum stimulation sessions, another after 6 verum stimulations, and the third after completing the first study cycle (sham). All other patients (n = 55) received 6 verum and 6 sham treatments and completed the CERAD for all required study visits. In 10 patients, at least 1 assessment occurred outside the designated visit window (±7 days around the scheduled appointment). The remaining 45 patients constitute the PP sample (23 verum-sham, 22 sham-verum).

Patients in the ITT sample (60; 30 females, 30 males) were a mean (SD) age of 70.65 (8.16) (range, 51-82) years and had 13.28 (3.44) years of formal education. Age was considerably older in the sham-verum group (mean [SD], 73.47 [6.59] years) compared with the verum-sham group (67.83 [8.69] years). The mean MMSE score was 23.77 (3.53), with most patients (n = 46) having mild dementia (MMSE, 18-26,), 5 patients showing moderate dementia (MMSE, 10-17), and 11 patients displaying mild or minor cognitive impairment (MMSE, 27-30). The BDI-II score at baseline was a mean of 7.17 (5.59), with most patients (n = 37) showing no depressive symptoms (BDI-II, 0-8), 15 patients with minimal depression (BDI-II, 9-13), 5 patients with mild depression (BDI-II, 14-19), 1 patient with severe depression

Figure 1. Trial Profile



Of 143 individuals screened for eligibility, 60 were randomly allocated to the treatment sequence groups verum followed by sham and sham followed by verum. In total, 60 patients were included in the intention-to-treat (ITTT) sample and 45 in the per-protocol (PP) sample. CTS indicates corrected total score.

(BDI-II, >29), and baseline BDI-II values missing for 2 patients. For more details, supplementary baseline characteristics, including patient medication are available in eTables 1-3 in Supplement 2.

Primary Outcome

The mean (SD) primary outcome CERAD CTS increased in the verum condition by 2.22 (6.87) points from 70.93 (14.27) at baseline to 73.15 (14.90) 3 months post stimulation, while the mean score in the sham condition increased by 1.00 (6.82) point from 71.68 (13.62) at baseline to 72.68 (14.48) 3 months post stimulation (**Table**; eFigure 4, and eTable 4 in Supplement 2). The rmANOVA revealed an effect of time (main effect of session: $P = .007$; $\eta p^2 = 0.07$) (eTable 5 in Supplement 2), but the interaction condition × session was not significant ($P = .68$; $\eta p^2 = 0.01$), in accordance with the findings of the contract research organization (eTable 39 in Supplement 2). As age differed significantly between the treatment sequence groups (mean [SD] verum-sham: 67.83 [8.69]; sham-verum: 73.47 [6.59] years; $P = .007$; unpaired t test), age at baseline was included as a covariate in the statistical model.

The post hoc rmANCOVA revealed an effect of session ($P = .008$; $\eta p^2 = 0.07$) (eTable 6 in Supplement 2) and an interaction of condition × session × age ($P = .003$; $\eta p^2 = 0.08$) that was followed by subgroup analyses (rmANOVA with condition and session as within-patient factors) for patients younger and older than the mean age of 70.65 years to disentangle the 3-way interaction. For the younger participants (mean age, 62.69; range, 51-70 years), a significant interaction between condition × session ($P = .005$; $\eta p^2 = 0.16$) was found with significantly higher score gains for all follow-up sessions in the verum compared with the sham condition ($\eta p^2 > 0.20$ for all post hoc contrasts) (eTable 7, eTable 8, and eFigure 4 in Supplement 2). The CERAD CTS increased 3.91 (7.86) points 3 months post stimulation compared with baseline in the verum condition but decreased 1.83 (5.80) points during the sham cycle in the younger patients. For the older participants (mean age, 76.73; range, 71-82 years), only the main effect of the session was significant ($P = .01$; $\eta p^2 = 0.11$) (eFigure 4D, eTable 9 in Supplement 2).

Pearson correlation analysis between age and the CERAD CTS outcome ([verum 3 months post stimulation vs verum baseline] vs [sham 3 months post stimulation vs sham baseline]) revealed a significant negative correlation ($r = -0.409$; $P = .001$), meaning that younger age was associated

Table. Neuropsychological Outcomes at Each Time Point in Intention-to-Treat Analysis

		Verum, mean (SD)				Sham, mean (SD)			
Test	No. of patients	Baseline	Post stimulation	1 mo Post stimulation	3-mo Post stimulation	Baseline	Post stimulation	1-mo Post stimulation	3-mo Post stimulation
CERAD CTS	60	70.93 (14.27)	72.69 (13.03)	72.92 (14.50)	73.15 (14.90)	71.68 (13.62)	72.26 (14.09)	73.00 (15.26)	72.68 (14.48)
NTBV	60	−1.65 (0.91)	−1.59 (0.89)	−1.56 (0.94)	−1.56 (0.93)	−1.66 (0.90)	−1.63 (0.95)	−1.56 (0.95)	−1.62 (0.97)
ADAS	60	19.40 (7.26)	19.10 (7.41)	18.97 (6.95)	18.48 (6.71)	19.77 (7.91)	19.10 (7.02	18.50 (7.44)	19.02 (7.21)
ADAS-Cog	60	16.77 (6.38)	17.18 (6.97)	16.70 (6.22)	16.25 (6.03)	17.38 (7.15)	16.68 (6.24)	16.37 (6.89)	16.83 (6.63)
CDT	60	4.72 (2.14)	4.63 (2.20)	4.85 (2.17)	4.75 (2.05)	4.83 (1.84)	4.88 (2.18)	4.80 (1.98)	4.78 (2.03)
FAI self	54	2.78 (0.75)	2.67 (0.74)	2.53 (0.79)	2.57 (0.73)	2.60 (0.70)	2.76 (0.75)	2.53 (0.76)	2.59 (0.78)
FAI ext	60	3.12 (0.80)	3.12 (0.86)	3.10 (0.87)	3.23 (0.94)	3.17 (0.84)	3.01 (0.88)	3.10 (0.87)	3.18 (0.94)
BDI-II	58	6.79 (5.25)	6.10 (5.27)	4.72 (4.06)	5.31 (4.96)	6.36 (5.56)	5.60 (5.28)	5.10 (5.26)	5.72 (4.53)
GDS	59	2.19 (1.72)	2.00 (1.91)	1.47 (1.47)	1.78 (2.25)	1.98 (1.95)	1.81 (2.13)	1.76 (1.99)	1.83 (2.17)
IADL self	60	0.91 (0.15)	0.92 (0.13)	0.93 (0.13)	0.89 (0.15)	0.93 (0.12)	0.93 (0.13)	0.92 (0.12)	0.93 (0.10)
IADL ext	60	0.80 (0.20)	0.82 (0.20)	0.81 (0.21)	0.81 (0.21)	0.80 (0.20)	0.81 (0.20)	0.83 (0.17)	0.80 (0.20)
B-ADL self	54	2.43 (1.40)	2.16 (1.21)	2.07 (0.98)	2.22 (1.11)	2.12 (1.02)	2.18 (1.01)	2.01 (0.96)	2.12 (1.18)
B-ADL ext	60	4.00 (1.95)	4.17 (2.08)	4.25 (2.15)	4.46 (2.27)	3.91 (1.89)	4.06 (1.91)	4.06 (1.96)	4.35 (2.17)
LB self	54	1.82 (0.58)	1.78 (0.58)	1.83 (0.58)	1.90 (0.59)	1.89 (0.62)	1.91 (0.60)	1.92 (0.65)	1.82 (0.61)
LB ext	60	1.66 (0.51)	1.62 (0.45)	1.67 (0.56)	1.63 (0.55)	1.65 (0.46)	1.71 (0.51)	1.60 (0.52)	1.60 (0.50)
NPI	60	6.67 (10.86)	7.27 (11.05)	6.42 (8.51)	7.00 (8.60)	5.77 (7.52)	6.93 (8.68)	6.08 (7.27)	6.50 (8.17)
PI	60	2.06 (0.88)	1.99 (0.98)	2.11 (0.88)	2.07 (0.91)	1.89 (0.85)	2.10 (0.93)	1.90 (0.85)	1.93 (0.87)

Abbreviations: ADAS, Alzheimer Disease Assessment Scale; ADAS-Cog, Alzheimer Disease Assessment Scale–Cognitive Scale; B-ADL, Bayer Activities of Daily Living Scale; BDI-II, Beck Depression Inventory-II; CDT, clock drawing test; CTS, corrected total score; ext, external assessment; FAI, Forgetfulness Assessment Inventory; GDS, Geriatric Depression Scale; IADL, Instrumental Activities of Daily Living Scale; LB, leisure behavior; NPI, Neuropsychiatric Inventory; NTBV, Neuropsychological Test Battery Vienna; PI, Prosopagnosia Index.

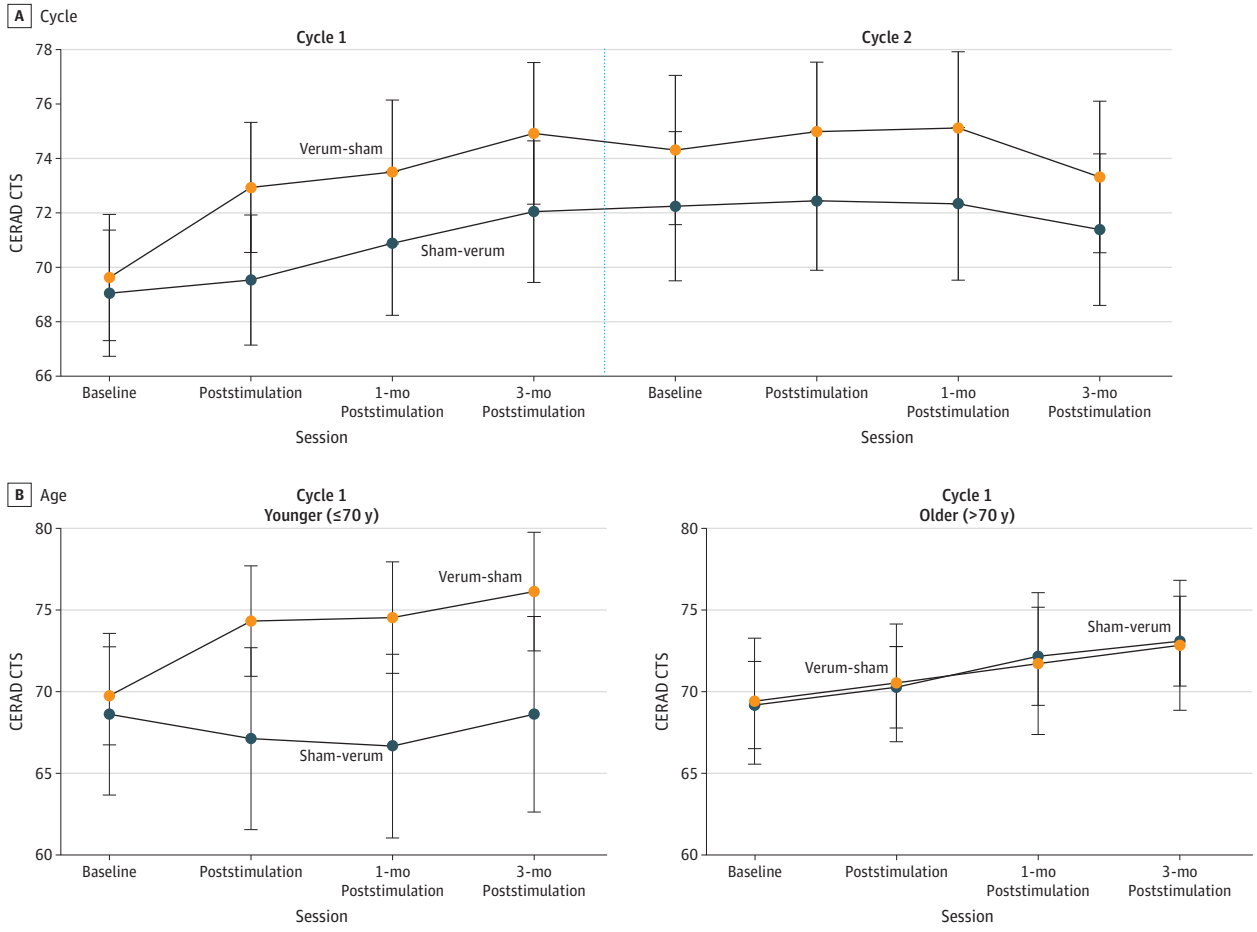
with better sham-controlled verum response. Yet, several patients older than 70 years benefited from verum treatment as well (eFigure 4 in Supplement 2).

Score increases were higher in the first cycle (particularly in the verum-sham group) compared with the second cycle, suggesting the presence of a carryover effect (**Figure 2**). To address this potential confounding, a post hoc ANCOVA of the first cycle only was conducted, with the between-patient factor group (reflecting verum and sham), session as within-patient factor, and age as covariates. While the analysis over all patients revealed a main effect of session only ($P < .001$; $\eta p^2 = 0.14$) (eTable 10 and eTable 11 in Supplement 2), a significant interaction between session × group ($P = .03$; $\eta p^2 = 0.12$) emerged for the younger patients, with significantly higher score increases in all poststimulation sessions for patients receiving verum compared with sham (ηp^2 >0.14 for all post hoc contrasts) (eTable 12 in Supplement 2; Figure 2). For the older patients, only an effect of session was found ($P = .02$; $\eta p^2 = 0.10$) (eTable 13 in Supplement 2). Results for the PP sample were similar to the findings for the ITT group (eTables 14-23 in Supplement 2).

fMRI Outcomes

Comparing the conditions with respect to changes in memory-associated brain activation from baseline (interaction condition × session), significantly higher posttreatment activation was

Figure 2. CERAD Corrected Total Scores (CTS)



CERAD CTS values over both cycles are plotted, with post hoc analysis of the first cycle (verum-sham) for the younger (age ≤70 years; n = 26 [19 in verum-sham; 7 in sham-verum]) and older (age >70 years; n = 34 [11 in verum-sham; 23 in sham-verum]) subsamples. A, CERAD CTS values show greater score increases in the group receiving verum transcranial pulse stimulation (TPS) in cycle 1 (verum-sham) and a sustained response suggesting a carryover effect. B, In the younger patients, a significantly greater improvement was found for all poststimulation sessions compared with the baseline in the group receiving verum compared with sham.

observed in the verum condition in the precuneus, a key area of the memory network,[22] visual areas, and in frontal regions supporting executive functions (superior and inferior frontal gyrus) (**Figure 3**). Whole-brain fMRI results are listed in eTable 40 in Supplement 2, with peak T value, cluster size, and Montreal Neurological Institute coordinates (eFigure 3 and eFigure 5 in Supplement 2). No area showed higher activation in the sham compared with the verum condition (Figure 3; eFigure 5 in Supplement 2). A supplementary analysis of memory-related brain activation within a bilateral parahippocampal region of interest revealed an effect of condition, with higher mean T values observed in the verum condition (eFigure 6 in Supplement 2). Higher mean T values within this region of interest were associated with fewer difficulties in activities of daily living (Bayer Activities of Daily Living external: ρ = −0.16; *P* = .02) and reduced forgetfulness (Forgetfulness Assessment Inventory external: ρ = −0.17; *P* = .01), as reported by the accompanying persons.

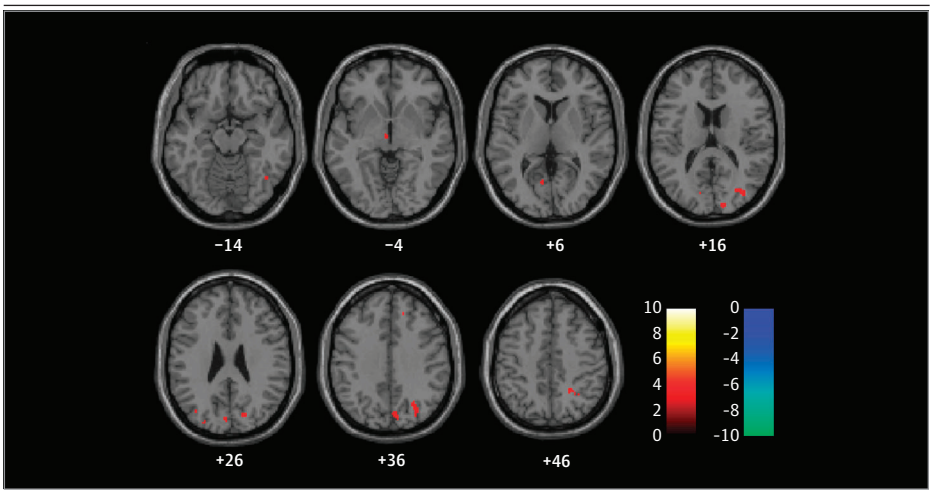
Besides main effects of condition in several cognitive networks (eTable 41 and eFigure 7 in Supplement 2), the GE analysis of resting-state fMRI data revealed a significant condition × session interaction in the dorsal attention network (*P* = .03). These effects specifically concerned the left frontal part of the dorsal attention network (*P* < .05), with increased GE compared with the respective baseline (eFigure 8 in Supplement 2). A sham vs verum GE was found in none of the networks investigated.

Secondary Behavioral Outcomes

Descriptive data for neuropsychological secondary outcomes are provided in the Table and detailed statistical reports are presented in eTables 24-29 (Supplement 2). For the self-reported leisure activities and the prosopagnosia index, a significant benefit for the verum condition was found.

Analyses for the PP sample were mostly consistent with ITT results (eTables 30-35 in Supplement 2), with one major exception. For the BDI-II, significant interactions of condition × session (*P* = .04; ηp^2 = 0.07) and condition × session × age (*P* = .008; ηp^2 = 0.11) emerged, with significantly lower BDI-II scores 3 months post stimulation compared with baseline in the verum condition (mean [SD] verum baseline: 7.27 [5.87]; verum 3 months post stimulation: 5.27 [5.27]; sham baseline: 6.70 [5.65]; sham 3 months post stimulation: 6.22 [4.40]; *P* = .008; ηp^2 = 0. 23). Separate ANOVAs for both age groups showed a significant interaction condition × session for both groups (younger: *P* = .04; ηp^2 = 0.19; older: *P* = .007; ηp^2 = 0.17) (eTables 36-38 in Supplement 2) with a nonsignificant improvement post stimulation in the younger participants and a significant reduction of depressive symptoms 3 months post stimulation in the older group (*P* = .02 with correction; ηp^2 = 0.28) (**Figure 4**). For the other neuropsychological secondary outcomes, no statistical significance was observed for the interactions of interest (condition × session, condition × session × age).

Figure 3. Functional Magnetic Resonance Imaging Results (fMRI)



For the fMRI memory task, the interaction condition (verum vs sham) × session (post stimulation vs baseline) revealed increased activation in the precuneus and frontal and visual areas following verum transcranial pulse stimulation (intention-to-treat sample, *P* = .001; uncorrected, k = 10).

Adverse Events

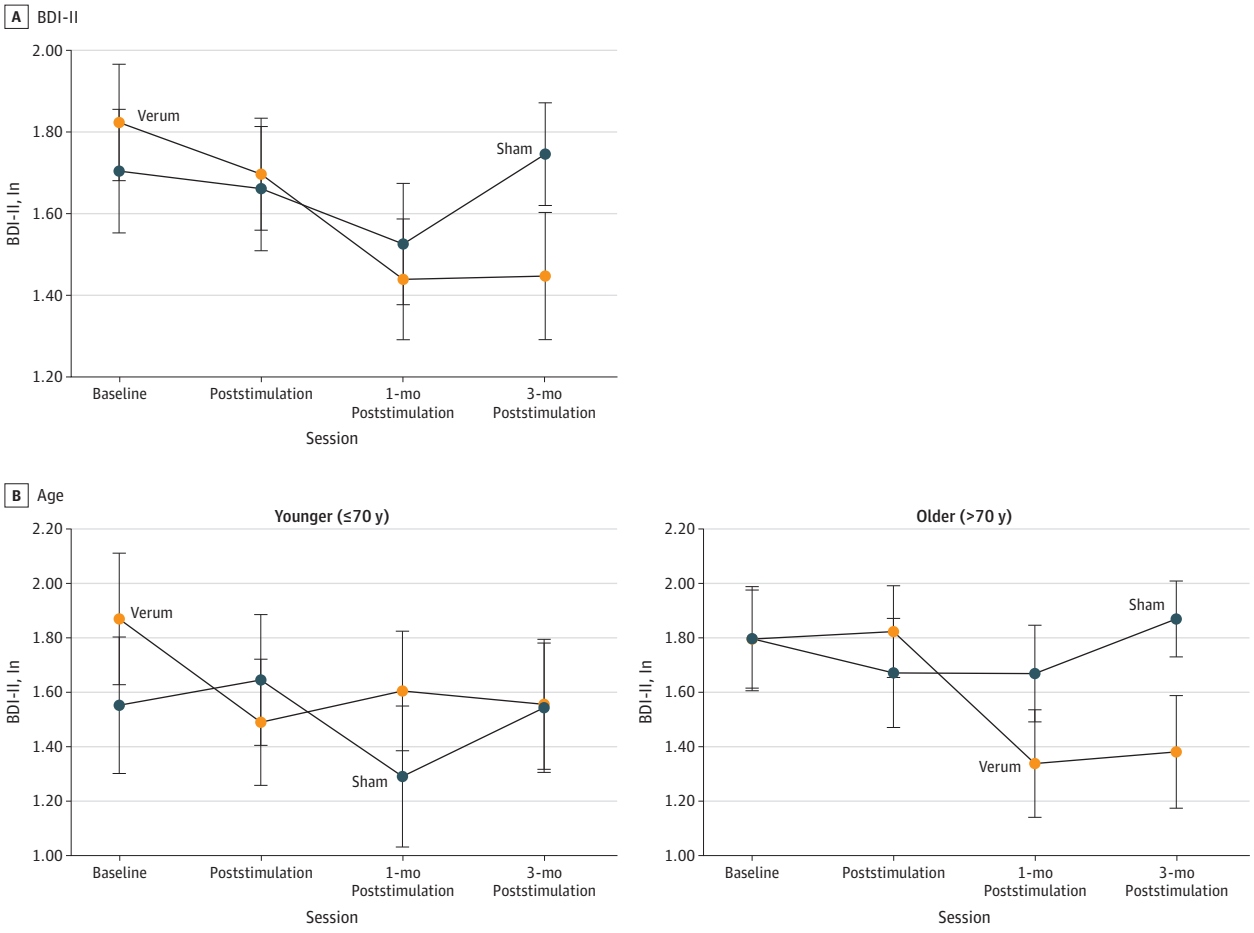
In total, 39 AEs with mild to moderate severity were noted in 24 participants (eTable 42 in Supplement 2). The most commonly observed symptom was depression, with clinically significant increases in depression scores (BDI-II) in 7 participants (12%), including 3 patients with a temporal association between symptom onset and verum TPS. A mild transient ischemic attack occurred in a participant during the washout period after completing the first cycle with sham applications. Further reports of AEs comprised headaches (7%); pain in the neck, shoulder, back, or hand (7%); dizziness (5%); anxiety (2%); sleep disorder (2%); and fatigue (2%), which were potentially related to general study procedures. No major neuropathologic change was detected in the patients by detailed neuroradiologic assessments, including the Fazekas score (eTable 43 in Supplement 2).

Subjective evaluations following the TPS interventions revealed low ratings of pain (<1 of 10) and pressure (<2 of 10). Accuracy for detecting the true condition was by chance, implying that blinding was successful (eTable 44 in Supplement 2).

Discussion

To our knowledge, this is the first randomized, sham-controlled clinical trial probing the effects of focused ultrasound neuromodulation in AD. We found increased memory-related brain activation

Figure 4. Beck Depression Inventory (BDI-II) Scores in the Per Protocol Sample



The logarithmically transformed BDI-II scores are shown for the verum and sham conditions for the per protocol sample (n = 37) (A), as well as for the younger (age ≤70 years, n = 14) and older (age >70 years, n = 23) subsamples (B). Symptom decrease compared with the baseline was significantly greater in the verum condition over all patients for the 3-month follow-up and for the 3-month follow-up for the older patients. Error bars indicate ±1 SE.

and functional connectivity compared with sham stimulation that translated to significant cognitive improvements in the younger subsample.

In more detail, a significant improvement over time was found for the primary outcome CERAD CTS, with increased scores in all poststimulation sessions compared with the baseline. Yet, a specific improvement for the verum compared with the sham condition was not significant and was influenced by the different age structure in the treatment sequence groups. By splitting the sample into a younger (aged ≤70 years) and older subsample, a significant benefit of verum TPS on the CERAD CTS was found for the younger patients.

Improvements in the CERAD CTS were primarily observed during the first cycle, with more-pronounced increases for verum TPS. The score appeared to plateau during the second cycle in both conditions, which diluted the overall increase in mean scores. In addition, different effects for verum and sham in the first cycle led to substantially different baseline levels between groups in the second cycle.

To address the bias induced by the carryover effect, a post hoc analysis of the first cycle only was conducted. With this analysis, CERAD CTS values improved by a mean (SD) of verum TPS (younger: 6.38 [7.51], older: 3.42 [6.91]), showing similar improvements as found in the pilot study.[14] Again, cognitive enhancement compared with sham was significant only in the younger subgroup, with large effects for all post hoc comparisons. Chronological age constitutes only an approximation for biological or brain age, which can be more informative for estimating treatment responses.[23]

In accordance with previous studies,[14-16,24] behavioral findings were supported by evidence from fMRI. Compared with sham, patients displayed significantly higher memory-related brain activation increase after verum stimulation in a key area of the memory network: the precuneus. Brain areas involved in executive and visual functions, as required in the face-name association task, also showed a significant verum TPS effect. Functional connectivity analyses revealed a significantly higher GE increase in the attention network for verum compared with sham, supporting an improvement of attentional aspects of cognition. In addition, fMRI data showed no area with a significantly higher memory-related activation or resting state GE in the sham condition, indicating a specific functional upregulation of cognitive areas and networks due to verum TPS.

Regarding depressive symptoms, a significant reduction in BDI-II scores was found for verum TPS in the PP sample 3 months following TPS treatment. This first observation of a sham-controlled antidepressant effect of TPS is in line with previous data in patients with AD[14,15,25,26] and in individuals with major depressive disorder.[27]

Besides the current investigation, to our knowledge, only 1 sham-controlled study of ultrasound neuromodulation in AD has been conducted, applying whole-brain stimulation using an unfocused diffusion type system in 15 patients with AD (10 verum, 5 sham).[28] While the verum group remained cognitively stable for over a year, as measured by the ADAS-cog (Japanese version), patients in the sham group exhibited cognitive decline. However, the group comparison did not reach statistical significance, consistent with the nonsignificant results for the ADAS-cog in this study. In other open-label ultrasound neuromodulation studies in AD, improved cognitive scores (eg, ADAS-cog) were observed.[25,26,29] Yet not all findings reached statistical significance, potentially due to the small sample size or the limited sensitivity of the cognitive tests used to detect mild deficits.

As the clinical application of ultrasound neuromodulation is still in its early stages,[7] the optimal settings and parameters remain to be established for maximizing efficacy. Based on our data and given the large interindividual variability, we suggest sample sizes exceeding 100 participants and longer follow-up periods to optimize future therapeutic research. Future clinical ultrasound neuromodulation studies should avoid the carryover effect by applying a parallel-group design or extended crossover intervals in clinically stable conditions. Comprehensive assessments of brain function, structure, and metabolism, alongside measurements of amyloid β and tau depositions, could provide deeper insights into the mechanisms of action of ultrasound neuromodulation in treating AD and other dementias.

Limitations

This study has limitations. By coincidence, the treatment sequence groups differed considerably regarding their age, with the group receiving verum TPS first being significantly younger. The neuropsychological tests were administered 8 times in total, so practice or attrition effects must be considered. Since learning curves are typically nonlinear and attenuated in older individuals or those with more impairments,[30] the potential bias of age had to be addressed by post hoc analyses. Future studies should prospectively consider age as factor, optimally combined with measures of biological and brain age.

Conclusions

In this randomized clinical trial of TPS in patients with AD, a 2-week treatment was well tolerated and improved cognitive scores (CERAD CTS) in the younger subgroup of patients with AD, ameliorated depressive symptoms, and induced upregulation of functional brain activation and connectivity. These findings suggest TPS as a safe and promising add-on therapy for patients with AD receiving state-of-the-art treatment.

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Trial Protocol

SUPPLEMENT 2.

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SUPPLEMENT 3.
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