



Functional Specificity of TPS Brain Stimulation Effects in Patients with Alzheimer's Disease: A Follow-up fMRI Analysis

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ABSTRACT

Introduction: Transcranial pulse stimulation (TPS) has been recently introduced as a novel clinical brain stimulation technique based on highly focused ultrasound pressure pulses. In a first pilot study on clinical effects of navigated and focused ultrasound neuromodulation, a dichotomy of functional effects was found: patients with Alzheimer's disease improved cognition and language but deteriorated with visuo-constructive functions.

Methods: We analyzed changes in functional connectivity measured with functional magnetic resonance imaging (fMRI) using graph analysis of a visuo-constructive network in 18 patients with Alzheimer's disease. We calculated the network's global efficiency and tested for correlation with visuo-constructive test scores to explain this dichotomy.

Results: Important visuo-constructive network nodes were not stimulated in the pilot setting and correspondingly global efficiency of a visuo-constructive network was decreased after

TPS therapy, compatible with a natural progress of the disease. A correlation between visuo-constructive scores and changes in global efficiency was found.

Conclusion: Results argue for a high functional specificity of ultrasound-based neuromodulation with TPS.

PLAIN LANGUAGE SUMMARY

Over the last decade, there has been growing interest in ultrasound-based non-invasive brain stimulation techniques in neuroscience and as a potential therapy for disorders of the brain. Transcranial pulse stimulation (TPS) has been introduced as an innovative neuromodulation technique, applying ultrashort pressure pulses through the skull into neural tissue with 3D navigation in real time. In the first clinical pilot study, patients suffering from Alzheimer's disease showed an increase in memory and language functions for up to 3 months after TPS therapy. However, visuo-constructive capacities (e.g., copying a geometrical figure) worsened. Notably, brain areas relevant for such processes had been left out during stimulation. This begged the question whether the brain areas that were targeted for brain stimulation as well as functional changes could explain this diverse response pattern. We therefore analyzed functional magnetic resonance data from patients.

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Specifically, we compared graph theoretical functional connectivity measures in a visuo-constructive network before and after TPS therapy. We found a decrease in connectivity in a central network node, which also correlated with visuo-constructive test scores. This deterioration is likely associated with normal disease progression. Together with the already reported improvement in global cognitive functions, these results argue for a functional specific effect of TPS.

Keywords: Alzheimer’s disease; Functional magnetic resonance imaging; Non-invasive brain stimulation; Transcranial pulse stimulation; Ultrasound; Visuo-construction

Key Summary Points

Why carry out this study?

A first pilot study investigating clinical effects of transcranial pulse stimulation (TPS) in patients with Alzheimer’s disease (AD) showed a split functional effect pattern, namely improved cognition and language but worsened visuo-constructive capacities.

This study investigated whether the targeted areas during brain stimulation and functional brain changes could illuminate these contrasting effects.

What was learned from this study?

Brain functions related to non-stimulated brain areas declined despite general cognitive improvement after AD stimulation therapy.

The non-stimulated brain areas concerned visuo-constructive processing, which declined according to neuropsychological scores. This decline corresponded to reduced connectivity of the visuo-constructive network.

These results underline the functional specificity of TPS with respect to the chosen stimulation targets.

INTRODUCTION

Recent developments in novel transcranial ultrasound techniques have enabled new possibilities for precise neuromodulation as well as clinical add-on treatment [1–3]. Advantages over electromagnetic techniques concern precise targeting independent from pathological brain conductivities and non-invasive stimulation deep in the brain [4–6]. Transcranial pulse stimulation (TPS) has been recently introduced as a novel clinical brain stimulation technique applying navigated ultrashort pressure pulses [7, 8]. Stimulation of a broad memory network in a group of patients with Alzheimer’s disease (AD) (including language areas and extended dorsolateral prefrontal cortex) exhibited functional as well as behavioral improvements in memory, language, and mood [9]. Furthermore, a reduction in cortical atrophy measures (increase of cortical thickness) in central AD network areas after TPS intervention has been shown [10]. Notably, while these improvements were observable for up to 3 months after stimulation, there was a deterioration of visuo-constructive capacities after TPS treatment. Visuo-constructive deficits are among the most common behavioral symptoms in different dementias [11]. While there is limited information from neuroimaging studies investigating neuronal correlates of constructional deficits, structural as well as metabolic reductions in the posterior and temporoparietal brain related to visuo-constructive processing have been shown in patients with dementia [12]. Serra et al. [13] described a visuo-constructive network (VisNW) in a comparison between patients exhibiting AD with and without constructional deficits. Importantly, these network areas have been largely left out during the multicentric clinical pilot TPS study, which could explain the visuo-constructive deteriorations. The goal of this follow-up analysis was to investigate whether the visuo-constructive decline could be supported by an objective neurophysiological measure—a network specific connectivity decrease measured by functional magnetic resonance imaging (fMRI).

METHODS

Patients

All patients with AD and available functional MRI data from our previous study [7] were reanalyzed ($n = 18$, 11 female, mean age 69.9 years, mean Mini-Mental State Examination (MMSE) score 20.94, standard deviation 5.8, range 6–30). Recruitment was performed by independent neurologists, following the diagnosis criteria given in the ICD-10 (F00) and the NIA-AA criteria by an expert in cognitive neurology. Only patients receiving already optimized standard treatments were accepted. Inclusion criteria were clinically stable patients with probable AD, at least 3 months of stable antedementia therapy, age at least 18, signed informed consent. Exclusion criteria were noncompliance with the protocol, relevant intracerebral pathology unrelated to AD, hemophilia or blood clotting disorders or thrombosis, and corticosteroid treatment within the last 6 weeks before treatment onset.

Ethics Statement

All procedures in this study were approved and performed in accordance with the ethical code of the Ethical Committee of the Medical University of Vienna (EK1227/2015) and the Declaration of Helsinki. Written, informed consent was obtained from all patients.

Study Design

Magnetic resonance imaging (MRI) data acquisition and neuropsychological tests were performed the week before and after patients received TPS treatment for 4 weeks, with three TPS sessions per week (three patients for 2 weeks, one patient for 3 weeks).

TPS Treatment and Regions of Interest

Single ultrasound pressure pulses were applied using a NEUROLITH TPS generator (Storz Medical AG, Tägerwilten, Switzerland): duration

about $3 \mu\text{s}$, 0.2 mJ mm^{-2} energy flux density, pulse repetition frequency 5 Hz, pulse number per therapeutic session 6000. Individual regions of interest (ROIs) were defined by a neurologist (R.B.) to target brain areas relevant to AD. These include the classical AD and depression stimulation target dorsolateral prefrontal cortex, areas of the memory (including default mode network), and language networks. Specifically, ROIs comprised bilateral frontal cortex (dorsolateral prefrontal cortex and inferior frontal cortex extending to Broca's area, ROI volume $136/164 \text{ cm}^3$, 2×800 pulses per hemisphere), bilateral lateral parietal cortex (extending to Wernicke's area, ROI volume $122/147 \text{ cm}^3$, 2×400 pulses per hemisphere), and extended precuneus cortex (one bilateral volume with $66/92 \text{ cm}^3$, 2×600 pulses). The goal was to distribute all pulses within the respective ROIs with a focus on the cortical tissue. Every ROI was stimulated twice per session. Two sets of standardized ROI sizes (ellipsoidal shape) were applied for either small or large sized patient brains (chosen according to pre-evaluation of brain size variability with in-house software for gross estimation of cerebrum size, based on their structural MRI). As previously described [7], individual real-time tracking allowed standardized focal brain stimulation across subjects.

MRI Parameters

T1-weighted structural images were recorded using a MPRAGE sequence (3 T Siemens PRISMA, TE/TR = 2.7/1800 ms, IT = 900 ms, FA = 9° , resolution 1 mm isotropic). Resting state fMRI covered the whole brain including cerebellum (GE-EPI sequence, 38 slices, TE/TR = 30/2500 ms, flip angle = 90° , in-plane acceleration = GRAPPA 2, field of view = $230 \times 230 \text{ mm}$, voxel size = $1.8 \times 1.8 \times 3 \text{ mm}$, 25% gap). A total of 250 volumes (10 min 25 s) were recorded.

Behavioral Assessment

Principle component analysis (PCA) from CERAD Plus (German version) scores [14] were generated using the z-transformed values

(corrected for age, gender, and formal education). The PCA on the CERAD subtests produced statistically independent factors that were able to explain individual test performance with an eigenvalue greater than 1, and therefore allowed grouping subtests according to cognitive components for memory, verbal processing, and visuospatial or constructional processing (FIGURAL) scores [7]. Specifically, the FIGURAL measure represented performance on a geometrical figure copy as well as a delayed figure recall task. Complete baseline and post-stimulation test data was available for 16 patients. FIGURAL test scores were normally distributed and further analyzed with paired *t* tests.

Preprocessing and Graph Analysis of a Visuo-constructive Network

Analysis was done with the CONN toolbox v19 [15]. Preprocessing comprised realignment, unwarping, slice-time correction, structural segmentation, normalization, and outlier detection (ART-based scrubbing). Following recommendation for fMRI group comparisons [16], images were smoothed with a kernel of 8 mm at full-width at half maximum, as the voxel size with gap was 3.75 mm. Subsequently, data was denoised using a band pass filter (0.008–0.09 Hz), removing motion confounds (six motion parameters and their first derivatives), applying aCompCor [17], and scrubbing. Bivariate correlations of the corrected time series of all voxels were calculated for first-level analysis. Graph theoretical measures were calculated within the a priori defined VisNW from Serra et al. [13]. The reported VisNW was largely in posterior and parietal brain areas and comprised the bilateral angular gyrus (AG), right intracalcarine cortex, right posterior medial temporal gyrus, posterior cingulate cortex, and right temporo-occipital fusiform cortex. These ROIs were defined in a nondirectional graph as nodes, with supra-threshold connections as edges. ROIs were taken from the Harvard–Oxford atlas. For each subject and condition, a graph adjacency matrix was then computed to calculate global efficiency (GE; a

global connectedness measure of each ROI within a network [18]) and compare between baseline and post-stimulation sessions (correlation coefficient = 0.35, *p*-FDR = 0.05, paired *t* test, two-sided). We chose GE because it is an established metric indicating the capacity to pass information in a network and therefore is of particular interest in our investigation. Individual GE values showing a significant difference between sessions were extracted and used to correlate (Spearman rank) with FIGURAL test scores.

RESULTS

Neuropsychological Assessment

Comparing CERAD FIGURAL test scores between pre and post stimulation using a paired *t* test showed a trend in declining visuo-constructive capabilities after TPS treatment (Fig. 1a; *p* = 0.0558). Notably, this decline was significant 3 months after stimulation (*p* = 0.007) [7].

Graph Analysis of Visuo-constructive Network and Correlation Analysis

Analyzing the visuo-constructive network revealed a significant decrease in GE for the VisNW (*p* = 0.016) with the right AG also showing an individual decline in GE (Fig. 2; *p*-FDR = 0.0153; corrected for multiple comparisons for each network node). CERAD FIGURAL test scores positively correlate with right AG GE values (*p* = 0.046; ρ = 0.356) and plotting the differences in individual GE values and corresponding FIGURAL scores together showed an overall non-significant trend of a joint decrease (Fig. 1b). This tendency is also reflected in the group mean. The VisNW as described by Serra et al. [13] also included parts of the precuneus, which, in fact, was among the targeted areas during TPS treatment. In the current analysis, this area was excluded to specifically test a network of nodes that have been left out during stimulation. However, we also tested the network including the precuneus

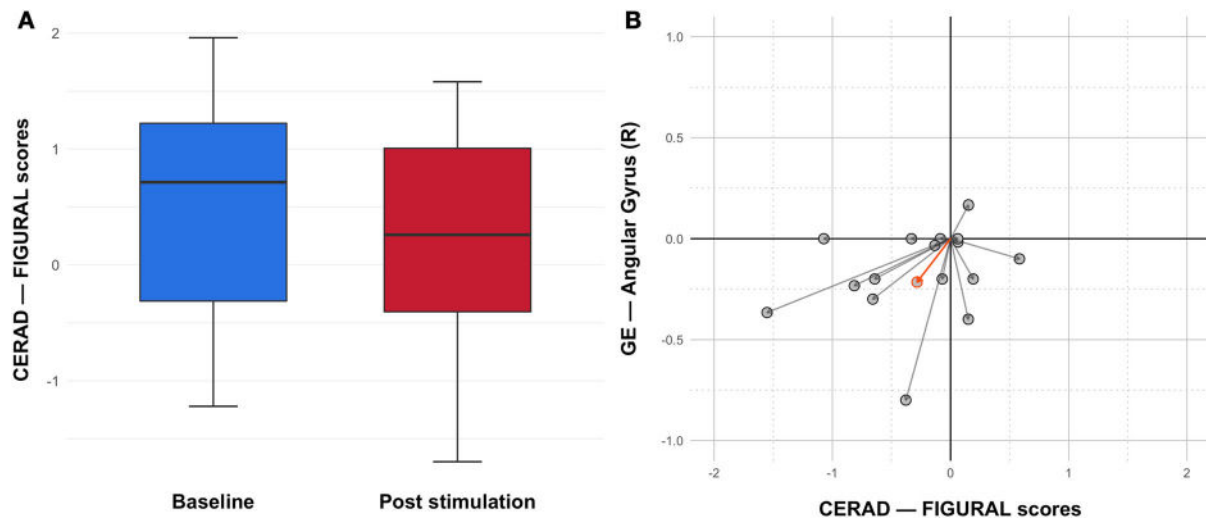


Fig. 1 Visuo-constructive performance decreases together with deterioration of functional connectivity. **a** CERAD FIGURAL test scores exhibit a trend to decline 1 week after the completed 4-week-long TPS treatment ($p = 0.0558$; paired t test, two-sided). This effect reached significance 3 months post treatment. **b** Individual

differences of FIGURAL test scores and global efficiency values of the right angular gyrus between baseline and post stimulation decreased together in the majority of patients. Arrows indicate changes for individual patients, with group mean difference in orange

and still found a significant GE decrease in the right AG (p -FDR = 0.0262), indicating a robust decline in network connectivity despite one node receiving stimulation.

DISCUSSION

The novel brain stimulation technique TPS can generate improvements for memory, language, and mood when targeting functionally relevant brain regions [7, 9]. In contrast, there was a specific decrease in visuo-constructive test scores while network areas for a VisNW had been omitted during stimulation. The present results support the decline in visuo-constructive abilities by the documented functional decrease in a VisNW, with a specific decrease in GE in the right AG. Owing to the high sensitivity of fMRI for detection of small functional brain changes, network changes were already significant immediately after the stimulation phase. Although a general correlation between visuo-constructive scores and network connectivity was found, analysis of individual pre-/post-treatment differences did not reach

significance, most likely because of our limited number of subjects. However, the group mean clearly supported this trend (Fig. 1b). Overall, the results indicate an associated functional and cognitive deterioration associated with brain areas that were not treated with TPS. Most likely this represents the natural progress of the disease [19].

The AG has been implicated in constructional processing [20–22], so a possible hypothesis is that because it is functionally more relevant in the VisNW than, e.g., the precuneus, network function decreased together with corresponding visuo-constructive capacities despite stimulation of the precuneus. Recently, TPS has been shown to induce long-term effects on functional and structural connectivity in connected network areas after stimulation of a somatosensory cortex area [23]. In a similar vein, Verhagen et al. [24] reported that in macaques not only stimulated brain regions but also their closest connected neighboring areas show modulated activity patterns. In contrast, networks functionally unrelated to the stimulation target did not exhibit significant changes, arguing for a functionally specific

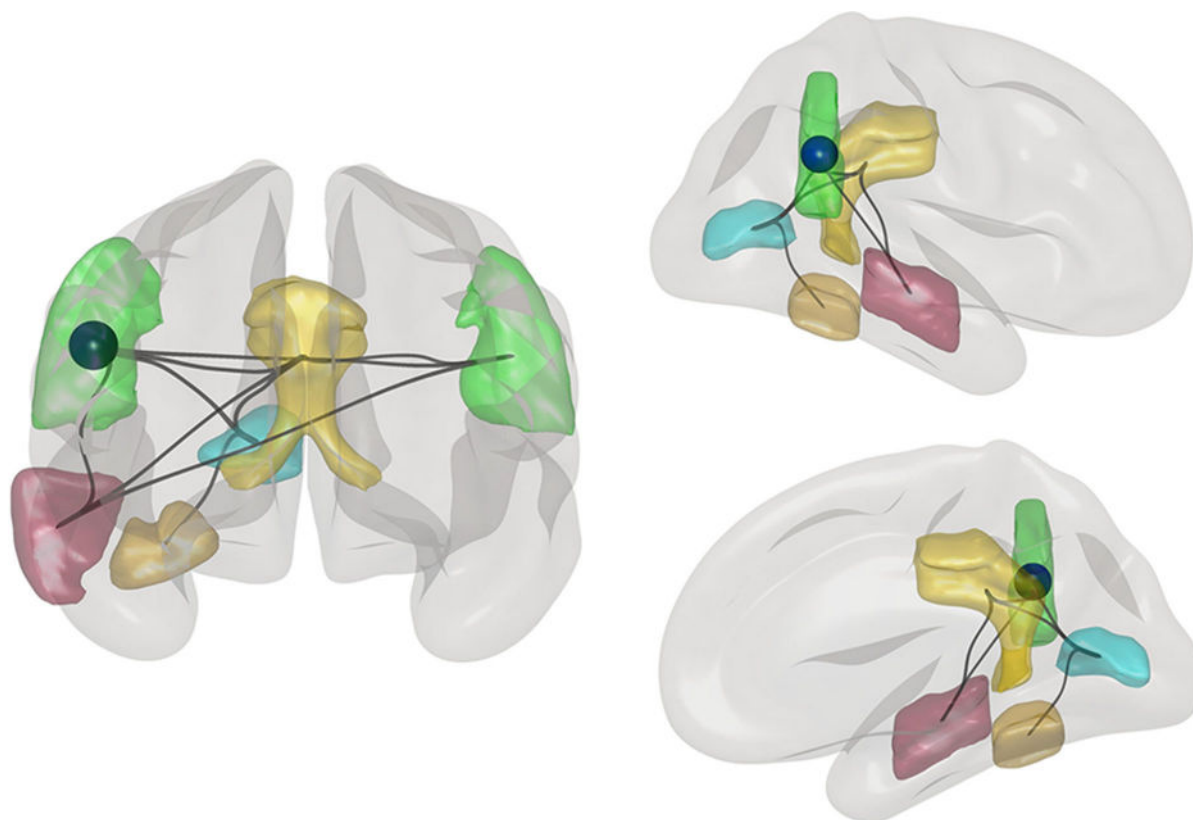


Fig. 2 Functional decline in a node of a visuo-constructive network. The global efficiency significantly decreased in the right angular gyrus (AG; indicated by the blue sphere) after TPS stimulation compared to the baseline. The visuo-constructive network consists of bilateral AG (green), right

intracalcarine gyrus (blue), posterior cingulate cortex (yellow), right posterior medio-temporal gyrus (red), and right temporo-occipital fusiform cortex (gold). Lines indicate all suprathreshold connections of the network

network effect. Together with previous results [7], the current study suggests, likewise, a confined, functionally specific response to TPS treatment with matching behavioral effects.

A number of limitations need to be addressed. There was no sham control included, and thus results need to be interpreted with care. However, as already discussed, the complex functional and behavioral responses shown here and elsewhere [7] argue against a global placebo response [25]. The small sample size limits any premature conclusions and larger studies are needed to elucidate individual treatment responses in more detail. Further, fMRI measurements were taken only at one time point about 5–6 weeks after the baseline fMRI, so longer-term fMRI behavior (more than 6 weeks) could not be investigated.

CONCLUSIONS

This study presents evidence for the spatial and functional specificity of ultrasound-based neuromodulation in a therapeutic context. Modern fMRI techniques allow one to elucidate the complex response patterns after TPS interventions, which will help to design stimulation protocols tailored to a specific clinical or neuroscientific interest. Future investigations may build on this functional specificity of TPS brain stimulation to develop optimized stimulation protocols also for visuo-constructive deficits in patients.

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Compliance with Ethic Guidelines. All procedures in this study were approved and performed in accordance with the ethical code of the Ethical Committee of the Medical University of Vienna (EK1227/2015) and the Declaration of Helsinki. Written, informed consent was obtained from all patients.

Data Availability. The datasets analyzed for the current study are not publicly available due to patient confidentiality and participant privacy restrictions.

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