The structural effects of transcranial magnetic stimulation on hippocampal subfields in Alzheimer's disease

Alzheimer hastalığında transkraniyal manyetik stimülasyonun hipokampal alt alanlar üzerindeki yapısal etkileri

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Abstract

Aim: This study investigates the structural effects of repetitive transcranial magnetic stimulation (rTMS) on hippocampal subfields and cortical shape metrics in Alzheimer's disease (AD) patients. Using high-resolution MRI segmentation and analysis via Hippunfold, we aim to elucidate TMS-induced structural changes and assess its potential neuroprotective role.

Methods: This retrospective study included 17 AD patients and 18 healthy controls (HC). AD patients underwent 20 Hz rTMS targeting the left lateral parietal cortex over 10 sessions across two weeks. Magnetic resonance imaging (MRI) data were acquired before and after rTMS and analyzed with Hippunfold to segment hippocampal subfields and extract cortical thickness and shape metrics. Statistical analyses were performed to compare subfield volumes and cortical metrics between groups and across time points.

Results: Hippocampal volumetric analysis revealed significant atrophy in subfields such as Cornu Ammonis 1, (CA1), CA2, CA4, dentate gyrus (DG), subiculum, and stratum radiatum-lacunosum-moleculare (SRLM) in AD patients compared to HC. Although no significant volumetric recovery was observed post-TMS, a further decline was noted in the right CA3 subfield ($p=0.005$), highlighting progressive atrophy. Cortical shape analyses showed significant reductions in hippocampal thickness ($p<0.001$) and surface area ($p<0.001$) in AD patients versus HC, with further cortical thinning in both hemispheres between pre- and post-TMS conditions. These findings suggest ongoing neurodegeneration despite TMS treatment.

Conclusion: TMS did not significantly reverse hippocampal atrophy or cortical thinning in this cohort. However, observed asymmetry in atrophy patterns, with relatively stable left hippocampal subfields compared to the right, suggests potential neuroprotective effects of TMS. These results highlight the need for prolonged and bilateral stimulation protocols to explore the therapeutic potential of TMS in mitigating AD progression.

Keywords: Alzheimer's disease; atrophy; hippokampus; transcranial magnetic stimulation

Öz

Amaç: Bu çalışma, Alzheimer hastalığı (AH) olan bireylerde tekrarlayan transkraniyal manyetik stimülasyonun (rTMS) hipokampal alt alanlar ve kortikal şekil metrikleri üzerindeki yapısal etkilerini araştırmaktadır. Yüksek çözünürlüklü MRI segmentasyonu ve Hippunfold analizi kullanılarak, TMS kaynaklı yapısal değişiklikleri incelemeyi ve TMS'nin olası nöroprotektif rolünü değerlendirmeyi amaçlıyoruz.

Yöntemler: Bu retrospektif çalışmada, 17 AH hastası ve 18 sağlıklı kontrol (SK) yer aldı. AH hastalarına, iki hafta boyunca toplam 10 seanslık sol lateral paryetal korteksi hedefleyen 20 Hz rTMS uygulandı. Tedavi öncesi ve sonrası MRI görüntüleri Hippunfold yazılımıyla analiz edilerek hipokampal alt alanlar segmentlendi ve kortikal kalınlık ile şekil metrikleri çıkarıldı. Gruplar arası ve zaman noktaları arasındaki karşılaştırmalar için istatistiksel analizler yapıldı.

Bulgular: Hipokampal volumetrik analiz, AH hastalarında Cornu Ammonis 1, (CA1), CA2, CA4, dentat girus (DG), subikulum ve stratum radiatum-lacunosum-moleculare (SRLM) gibi alt alanlarda belirgin atrofi olduğunu ortaya koydu. TMS sonrası anlamlı bir volumetrik iyileşme gözlenmese de, sağ CA3 alt alanında progresif atrofi tespit edildi ($p=0.005$). Kortikal şekil analizleri, AH hastalarında hipokampal kalınlıkta ($p<0.001$) ve yüzey alanında ($p<0.001$) sağlıklı kontrollere kıyasla anlamlı azalmalar olduğunu gösterdi ve her iki hemisferde de TMS öncesi ve sonrası arasında kortikal incelme görüldü. Bu bulgular, TMS tedavisine rağmen devam eden nörodejenerasyonu işaret etmektedir.

Sonuç: TMS, bu çalışmada hipokampal atrofiyi veya kortikal incelmeyi anlamlı şekilde tersine çevirmemiştir. Ancak, sol hipokampal alt alanların sağa göre daha stabil olması, TMS'nin potansiyel nöroprotektif etkilerini işaret etmektedir. Bu sonuçlar, TMS'nin AH progresyonunu hafifletme potansiyelini araştırmak için daha uzun süreli ve çift taraflı stimülasyon protokollerinin gerekliliğine dikkat çekmektedir.

Anahtar Sözcükler: Alzheimer hastalığı; atrofi; hipokampus; transkraniyal manyetik uyarı

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INTRODUCTION

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder that affects millions worldwide, posing a major health and social burden due to its progressive nature and irreversible cognitive decline. Characterized by memory loss, impaired executive function, and diminished daily functioning, AD primarily targets brain regions involved in memory and learning, particularly the hippocampus. The hippocampus is one of the earliest and most affected regions in AD, undergoing marked atrophy and cellular loss even in the initial stages of the disease. This atrophy is not uniform; it specifically impacts various hippocampal subfields, such as CA1, CA2, CA3, CA4, the dentate gyrus (DG), and the subiculum, which are each associated with different memory and cognitive functions (1). As the disease advances, structural deterioration in these subregions worsens, correlating strongly with the progressive cognitive decline observed in AD patients (2). Understanding the specific patterns of degeneration in hippocampal subfields may provide key insights into the progression of AD and help identify potential targets for intervention.

In recent years, Transcranial Magnetic Stimulation (TMS) has gained attention as a promising, non-invasive treatment modality for enhancing cognitive function in AD patients (3). TMS uses magnetic fields to stimulate neural activity, potentially altering brain circuits involved in cognitive processing. Studies suggest that TMS may offer neuroprotective benefits by promoting neural plasticity and reducing synaptic loss, thereby slowing the structural degradation associated with AD (4). Additionally, TMS has shown promise in modulating hippocampal activity, which could directly impact memory and cognitive functions (5–7). However, despite the potential of TMS, there is a lack of comprehensive studies examining its long-term structural effects on specific hippocampal subfields in AD. Understanding how TMS affects these subfields individually could clarify its potential as a targeted intervention in AD and offer insights into its underlying mechanisms.

In this study, we aim to investigate the impact of TMS on hippocampal subfield volumes in AD patients by employing Hippunfold, a cutting-edge software tool for high-resolution segmentation and analysis of hippocampal subfields (8). Hippunfold enables precise mapping of hippocampal regions, allowing us to assess structural changes with greater accuracy. By comparing MRI data from before and after TMS treatment in AD patients, alongside MRI data from age-matched healthy controls, we aim to elucidate any TMS-induced structural changes and evaluate their relevance in the context of AD. This approach not only enables us to assess the potential neuroprotective effects of TMS but also helps us identify disease-specific alterations in hippocampal subfields. We hypothesize that TMS treatment induces structural changes in the hippocampal subfields of AD patients, potentially preserving or enhancing the integrity of certain subfields compared to those of healthy controls. We further hypothesize that these changes will be more pronounced in regions typically affected by AD-related atrophy, such as the CA1 and DG subfields, thereby demonstrating the neuroprotective potential of TMS in mitigating hippocampal degeneration in AD.

MATERIALS AND METHODS

Participants

This retrospective study included 35 participants recruited from Istanbul Medipol University Mega Hospital, divided into two groups: 17 individuals diagnosed with Alzheimer's disease (mean age ± SD: 66.55 ± 5.52 years) and 18 age-matched healthy controls (mean age ± SD: 70.35 ± 7.82 years), with no significant age difference between the groups ($p = 0.109$). Alzheimer's disease was diagnosed based on standard clinical criteria, and all AD patients exhibited mild to severe cognitive impairment, as reflected by a Clinical Dementia Rating (CDR) score of 1 or higher. Each participant underwent a detailed medical history review, a thorough physical examination, and a cognitive assessment to ensure study eligibility and establish baseline cognitive status.

The Mini-Mental State Examination (MMSE) was administered to all participants as a measure of global cognitive function. AD patients exhibited MMSE scores consistent with their clinical diagnosis, ranging from mild to severe cognitive impairment, while healthy controls demonstrated normal cognitive function with no history of neurological or psychiatric

disorders. Healthy control participants were further screened to ensure they had no history of traumatic brain injury or neurological disease, allowing for a clear comparison between AD-affected and neurologically healthy brains.

This study was approved by Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (date: 03.12.2024, decision no: E-10840098-202.3.02-7360). All participants provided written informed consent prior to enrollment, in accordance with the Declaration of Helsinki.

TMS application

This study was conducted retrospectively, utilizing MRI data from before and after treatment of AD patients who had previously undergone repetitive transcranial magnetic stimulation (rTMS). The TMS protocol, as applied in our prior study (7), involved stimulation of the left lateral parietal cortex at a frequency of 20 Hz to target the left hippocampus indirectly, following a network-based targeting method (9). This approach leverages the connectivity between the parietal cortex and hippocampus, modulating hippocampal activity through its cortical network connections.

The rTMS treatment was administered over a two-week period, consisting of 10 daily sessions. Each session delivered a total of 1,640 magnetic pulses at an intensity of 100% of the patient's resting motor threshold to ensure effective cortical stimulation. MRI images were collected from each AD patient both before the initiation of TMS treatment and after the two-week intervention, allowing for pre- and post-intervention comparison of hippocampal subfield volumes.

Imaging acquisition

Structural and functional MRI data were acquired for each participant using a Philips Achieva 3 Tesla MRI scanner (Philips Medical Systems), ensuring high-resolution images suitable for detailed hippocampal subfield analysis. T1-weighted anatomical images were obtained to facilitate segmentation and volumetric analysis of hippocampal subregions.

The T1-weighted anatomical images were collected using high-resolution imaging parameters to enable detailed structural analysis. A total of 190 contiguous slices were acquired, covering a field of view (FOV) of 256 × 256 × 190 mm. The images were obtained with an isotropic voxel size of 1 × 1 × 1 mm, providing consistent spatial resolution across all axes. The imaging protocol used a repetition time (TR) of 8.1 ms, an echo time (TE) of 3.7 ms, and a flip angle of 8°, which together were optimized to enhance image clarity and structural delineation.

These imaging parameters were selected to optimize image quality, allowing for precise segmentation of hippocampal subfields and accurate assessment of structural integrity. The high isotropic voxel resolution (1 mm^3) facilitates detailed analysis of hippocampal morphology, which is essential for detecting subtle volumetric changes in AD-related atrophy and potential structural alterations due to TMS treatment.

Hippunfold analysis

Hippocampal subfields were segmented and analyzed using Hippunfold (version 1.3.x), a specialized software tool designed for the precise delineation of hippocampal subregions. Hippunfold enables high-resolution, automated segmentation of specific hippocampal subfields, including CA1, CA2, CA3, CA4, DG, subiculum, and the stratum radiatum-lacunosum-moleculare (SRLM).

Each participant's T1-weighted MRI images were input into the Hippunfold pipeline, executed by running the hippunfold command. This command utilizes advanced cortical unfolding algorithms to model and extract volumetric data from hippocampal subfields with high anatomical accuracy. The isotropic 1 × 1 × 1 mm resolution of the T1-weighted images was essential for reliable subfield segmentation, minimizing partial volume effects and ensuring high precision in volumetric measurements.

Upon completion, Hippunfold generated an output folder labeled anat, containing a file named desc-subfields_atlas-multihist7_volumes.tsv, which recorded the segmented left and right volumes of each hippocampal subfield. In addition to volumetric data, further cortical thickness and shape metrics were analyzed using files generated in the surf folder. Specific metrics included:

Left dentate gyrus metrics: L Dentate Gyrification shape, L Dentate Surfarea shape

Left hippocampus metrics: L Hipp Curvature shape, L Hipp Gyrification shape, L Hipp Surfarea shape, L Hipp Thickness shape

Right dentate gyrus metrics: R Dentate Gyrification shape, R Dentate Surfarea shape

Right hippocampus metrics: R Hipp Curvature shape, R Hipp Gyrification shape, R Hipp Surfarea shape, R Hipp Thickness shape

Scalar metrics for the dentate gyrus and hippocampus: Dentate Curvature dscalar, Dentate Gyrification dscalar, Hipp Curvature dscalar, Hipp Gyrification dscalar, Hipp Thickness dscalar

These surface-based metrics provided additional insights into cortical remodeling beyond volumetric changes, capturing features such as thickness, surface area, curvature, and gyrification. By comparing these metrics across pre-TMS and post-TMS scans of AD patients and the healthy control group, the analysis allowed for a more comprehensive evaluation of structural differences and potential effects of TMS on hippocampal architecture.

Following segmentation, thickness, and shape metric extraction, the data were statistically analyzed to identify significant differences between pre-, and post-TMS scans and between AD and control groups. These comparisons offered a detailed assessment of subfield-specific changes, cortical remodeling, and the potential structural impact of TMS on hippocampal subfields in AD.

Statistical analysis

Statistical analyses were conducted to evaluate group differences in hippocampal subfield volumes, with all analyses performed using Jamovi software (version 2.3.21.0) and a significance threshold set at $p<0.05$. Group comparisons included: (1) Healthy Controls vs. Pre-TMS AD, (2) Healthy Controls vs. Post-TMS AD, and (3) Pre-TMS vs. Post-TMS AD. Independent (unpaired) t-tests were used to assess differences in hippocampal subfield volumes between healthy controls and the AD groups (pre- and post-TMS), as these comparisons involved separate groups. For comparisons within the AD group (pre- vs. post-TMS), paired t-tests were employed to account for the repeated measures design and to evaluate changes in hippocampal subfield volumes following TMS treatment. In addition to p-values, t-statistic values were reported to provide further insight into the magnitude of group differences. To minimize inter-individual variability, volumetric data were standardized by dividing each subfield volume by the total hippocampal volume (sum of all seven subfields). This statistical approach enabled precise evaluation of TMS-induced changes in hippocampal subfields and facilitated a robust comparison with healthy controls, allowing us to assess the potential structural effects of rTMS treatment in Alzheimer's patients.

RESULTS

Hippunfold analysis revealed significant atrophy in several left hippocampal subfields in AD patients compared to healthy controls HC. The subiculum volume was significantly reduced in pre-TMS ($p<0.001$) and post-TMS ($p<0.001$) AD groups compared to HC. Similarly, the CA1 subfield exhibited significant reductions in pre-TMS ($p=0.001$) and post-TMS ($p<0.001$) conditions relative to HC. Significant reductions were also observed in the CA3 ($p<0.001$), CA4 ($p<0.001$), DG (p $p<0.001$), and SRLM ($p<0.001$) subfields in pre-TMS and post-TMS AD groups compared to HC. However, no significant changes in volumetric measures were observed between pre-TMS and post-TMS conditions for any of these subfields.

In the right hippocampus, similar patterns of atrophy were observed in AD patients. The subiculum and CA1 subfields were significantly reduced in both pre-TMS ($p<0.001$) and post-TMS ($p<0.001$) conditions compared to HC. The CA3 subfield showed progressive atrophy, with significant reductions in AD patients compared to HC (pre-TMS: $p=0.003$; post-TMS: $p<0.001$) and a further significant decrease between pre-TMS and post-TMS conditions ($p=0.005$). Significant reductions were also found in CA4 ($p<0.001$) and DG ($p<0.001$) volumes in pre-TMS and post-TMS AD groups compared to HC, but no significant differences were observed between pre-TMS and post-TMS conditions. The SRLM subfield was also significantly reduced in AD patients relative to HC ($p<0.001$), with no evidence of recovery post-TMS (Table 1 Fig. 3).

Cortical shape and thickness analyses revealed sig-

Table 1. Mean hippocampal subfield volumes (± SD) in healthy controls (HC), pre-TMS AD patients, and post-TMS AD patients.

Items	HC	pre TMS	post TMS	HC vs pre TMS	HC vs post TMS	pre TMS vs post TMS
	Mean ± SD	Mean ± SD	Mean ± SD	p (t)	p (t)	p (statistic)
L Sub	583.6 ± 86.1	412.1 ± 79.1	393.1 ± 68.3	<0.001 (6.143)	<0.001 (7.27)	0.225 (103)
L CA1	763.9 ± 95.9	573.0 ± 169.4	564.4 ± 138.8	0.001 (4.072)	<0.001 (4.92)	0.644 (87)
L CA2	131.4 ± 24.2	107.9 ± 40.7	108.7 ± 28.7	0.116 (2.068)	0.044 (2.52)	0.927 (74)
L CA3	208.8 ± 39.5	141.7 ± 48.4	151.5 ± 40.0	<0.001 (4.472)	<0.001 (4.26)	0.611 (65)
L CA4	271.5 ± 40.2	195.9 ± 53.4	202.1 ± 60.8	<0.001 (4.714)	0.001 (3.96)	0.378 (57)
L DG	126.9 ± 17.6	64.4 ± 30.7	65.4 ± 29.9	<0.001 (7.323)	<0.001 (7.35)	0.963 (78)
L SRLM	560.0 ± 67.5	359.3 ± 85.2	358.0 ± 92.8	<0.001 (7.69)	<0.001 (7.33)	0.611 (88)
R Sub	566.4 ± 79.7	387.5 ± 108.1	406.1 ± 112.2	<0.001 (5.548)	<0.001 (4.85)	0.109 (42)
R CA1	809.8 ± 95.5	607.5 ± 138.0	601.7 ± 145.2	<0.001 (5.015)	<0.001 (4.98)	0.207 (104)
R CA2	139.8 ± 19.6	122.0 ± 36.3	131.5 ± 40.5	0.195 (1.786)	0.728 (0.764)	0.89 (73)
R CA3	246.0 ± 40.1	185.6 ± 55.2	169.4 ± 52.5	0.003 (3.684)	<0.001 (4.83)	0.005 (134)
R CA4	259.5 ± 46.6	189.1 ± 48.8	186.6 ± 62.9	<0.001 (4.361)	0.002 (3.88)	0.89 (73)
R DG	134.3 ± 17.5	65.7 ± 32.5	63.2 ± 35.7	<0.001 (7.71)	<0.001 (7.42)	0.306 (99)
R SRLM	593.3 ± 73.2	371.8 ± 105.9	367.5 ± 113.0	<0.001 (7.156)	<0.001 (6.97)	0.353 (97

Volumetric differences between groups were analyzed using independent t-tests (Healthy Controls [HC] vs. pre-Transcranial Magnetic Stimulation [TMS] Alzheimer's Disease [AD] patients and HC vs. post-TMS AD patients) and paired t-tests (pre-TMS vs. post-TMS). The hippocampal subfields assessed include the left and right subiculum (L Sub, R Sub), CA1 (Cornu Ammonis 1; L CA1, R CA1), CA2 (L CA2, R CA2), CA3 (L CA3, R CA3), CA4 (L CA4, R CA4), dentate gyrus (L DG, R DG), and stratum radiatum-lacunosum-moleculare (L SRLM, R SRLM). Each cell presents the mean volume with standard deviation (Mean ± SD) followed by the p-value and t-statistic (p(t)) for each comparison. Significant p-values ($p < 0.05$) indicate notable differences in subfield volumes across conditions, highlighting potential TMS-induced structural changes in AD patients.

Table 2. Comparisons of cortical shape and thickness metrics between HC, pre-TMS, and post-TMS AD groups

Items	HC	pre TMS	post TMS	HC vs. pre TMS		HC vs. post TMS	
				Statistic	p	Statistic	p
L Dentate Gyrification shape	5.9553 ± 0.9931	4.2351 ± 1.5254	4.7 ± 1.7803	60	0.002	73	0.007
L Dentate Surfarea shape	0.1317 ± 0.0125	0.1009 ± 0.0165	0.1021 ± 0.0189	18	< 0.001	17	< 0.001
L Hipp Curvature shape	-0.0922 ± 0.0126	0.062 ± 0.7224	-0.0865 ± 14.2712	119	0.273	118	0.258
L Hipp Gyrification shape	2.133 ± 0.1845	1.7314 ± 0.3042	1.6633 ± 0.2916	43	< 0.001	34	< 0.001
L Hipp Surfarea shape	0.1515 ± 0.0126	0.1176 ± 0.0163	0.1153 ± 0.0161	13	< 0.001	12	< 0.001
L Hipp Thickness shape	1.3418 ± 0.0504	1.1711 ± 0.1506	1.1692 ± 0.1378	28	< 0.001	28	< 0.001
R Dentate Gyrification shape	6.1167 ± 1.1562	4.041 ± 1.5756	3.561 ± 1.5304	49	< 0.001	39	< 0.001
R Dentate Surfarea shape	0.1329 ± 0.0141	0.0986 ± 0.0177	0.0953 ± 0.0201	24	< 0.001	26	< 0.001
R Hipp Curvature shape	0.0841 ± 0.0173	2.6782 ± 12.4624	0.087 ± 0.0195	125	0.369	143	0.757
R Hipp Gyrification shape	2.229 ± 0.2136	1.7665 ± 0.3435	1.7661 ± 0.3115	41	< 0.001	32	< 0.001
R Hipp Surfarea shape	0.1582 ± 0.0126	0.1244 ± 0.0244	0.1175 ± 0.0204	40	<0.001	29	< 0.001
R Hipp Thickness shape	1.3399 ± 0.0516	1.1811 ± 0.1224	1.1883 ± 0.1095	39	< 0.001	20	< 0.001
Dentate Curvature dscalar	-0.0041 ± 0.0088	1.37 ± 6.5880	1.55 ± 7.1380	136	0.59	125	0.369
Dentate Gyrification dscalar	6.0360 ± 1.0406	4.14 ± 1.3250	4.12 ± 1.4610	42	< 0.001	42	< 0.001
Hipp Curvature dscalar	-0.0041 ± 0.0088	1.37 ± 6.5880	1.55 ± 7.1380	136	0.59	125	0.369
Hipp Gyrification dscalar	2.1810 ± 0.1758	1.75 ± 0.2920	1.73 ± 0.2740	39	< 0.001	26	< 0.001
Hipp Thickness dscalar	1.3409 ± 0.0458	1.18 ± 0.1270	1.17 ± 0.1160	29	< 0.001	20	< 0.001

The table presents the mean ± standard deviation (SD) for Healthy Controls (HC), pre-Transcranial Magnetic Stimulation (TMS) Alzheimer's Disease (AD) patients, and post-TMS AD patients across various metrics, including the dentate gyrus and hippocampal gyrification, surface area, curvature, and thickness. Statistical comparisons were performed to evaluate differences between HC vs. pre-TMS, HC vs. post-TMS, and pre-TMS vs. post-TMS groups. Significant results ($p < 0.05$) are highlighted. The metrics include hemisphere-specific measures (e.g., L Dentate Gyrification for the left dentate gyrus) as well as scalar metrics such as hippocampal thickness (Hipp Thickness dscalar) and dentate curvature (Dentate Curvature dscalar).

nificant differences between HC and AD patients, as well as between pre-TMS and post-TMS conditions in specific hippocampal subfields and cortical metrics.

For gyrification, surface area, and curvature measures, left dentate gyrus (L Dentate Gyrification shape) showed a significant reduction in pre-TMS (p=0.002) and post-TMS (p=0.007) AD groups compared to HC, while no significant changes were observed between pre-TMS and post-TMS conditions. Similarly, right dentate gyrus curvature (R Dentate Curvature shape) was significantly reduced in pre-TMS and post-TMS AD patients relative to HC (p<0.001), with no significant differences between pre- and post-TMS conditions. For surface area, significant reductions were also observed in both left hippocampal (L Hipp Surfacearea shape) and right hippocampal (R Hipp Surfacearea shape) metrics in AD patients compared to HC (p<0.001 for both).

In terms of hippocampal thickness, significant reductions were observed in the left hippocampal thickness (L Hipp Thickness shape) and right hippocampal thickness (R Hipp Thickness shape) in pre-TMS and post-TMS AD groups relative to HC (p<0.001). Notably, hippocampal thickness measures further declined significantly between pre-TMS and post-TMS conditions in both hemispheres (p<0.001), indicating progressive cortical thinning despite TMS treatment.

Finally, scalar measures of dentate gyrus curvature (Dentate Curvature dscalar) and hippocampal thickness (Hipp Thickness dscalar) showed significant reductions in AD patients compared to HC (p<0.001), with further reductions observed between pre-TMS and post-TMS conditions (p<0.001), emphasizing ongoing structural degeneration (Table 2).

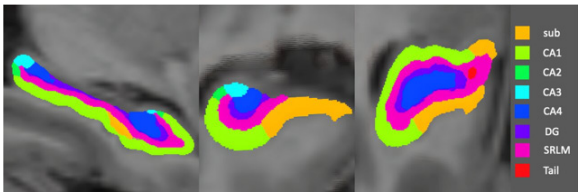

Figure 1. Segmented hippocampal subfields visualized on T1-weighted MRI images using the Hippunfold software. The image shows coronal, sagittal, and axial views of the hippocampus with color-coded labels for each subfield. Each color represents a distinct hippocampal subfield, facilitating detailed structural analysis and volumetric comparisons across different conditions. Sub: Subiculum, CA: Cornu Ammonis, DG: Dentate Gyrus, SRLM: Stratum Radiatum-Lacunosum-Moleculare, MRI: Magnetic Resonance Imaging

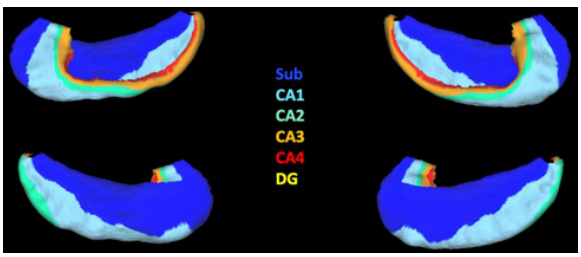

Figure 2. The figure depicts a 3D representation of hippocampal subfields segmented and color-coded using Hippunfold. Thickness is measured as the distance between the inner and outer surfaces of the hippocampal ribbon in each subfield. The visualization provides a clear spatial depiction of regional boundaries and structural properties, facilitating subfield-specific analyses of atrophy and cortical thinning in AD. Subfields are displayed bilaterally for both hemispheres, highlighting the utility of Hippunfold in quantifying structural metrics across the hippocampus. Sub: Subiculum, CA: Cornu Ammonis, DG: Dentate Gyrus, AD: Alzheimer's Disease

DISCUSSION

TMS and progression of atrophy

One notable observation was the persistent significant atrophy in most hippocampal subfields in AD patients compared to HC, particularly in the DG, CA1, CA3, CA4, and subiculum. Although rTMS did not cause volumetric recovery, the slight reduction in post-TMS comparisons (e.g. HC vs. post-TMS), which was not statistically significant, suggests that TMS may have modestly slowed the progression of hippocampal atrophy in certain regions. This subtle neuroprotective effect aligns with prior research suggesting that rTMS may promote neuroplasticity and enhance synaptic connectivity, potentially moderating the pace of structural degeneration (4). Long-term studies with extended TMS protocols are needed to clarify its effects on the trajectory of hippocampal atrophy.

Asymmetry in TMS effects: Left vs. Right hippocampus

The asymmetry in atrophy patterns between the left and right hippocampus was another key finding. TMS was administered to the left lateral parietal cortex, targeting the left hippocampus via network connectivity. Consequently, left hippocampal subfields, including the CA3, CA4, and DG exhibited a relatively stable profile compared to the right hippocampus, where at-

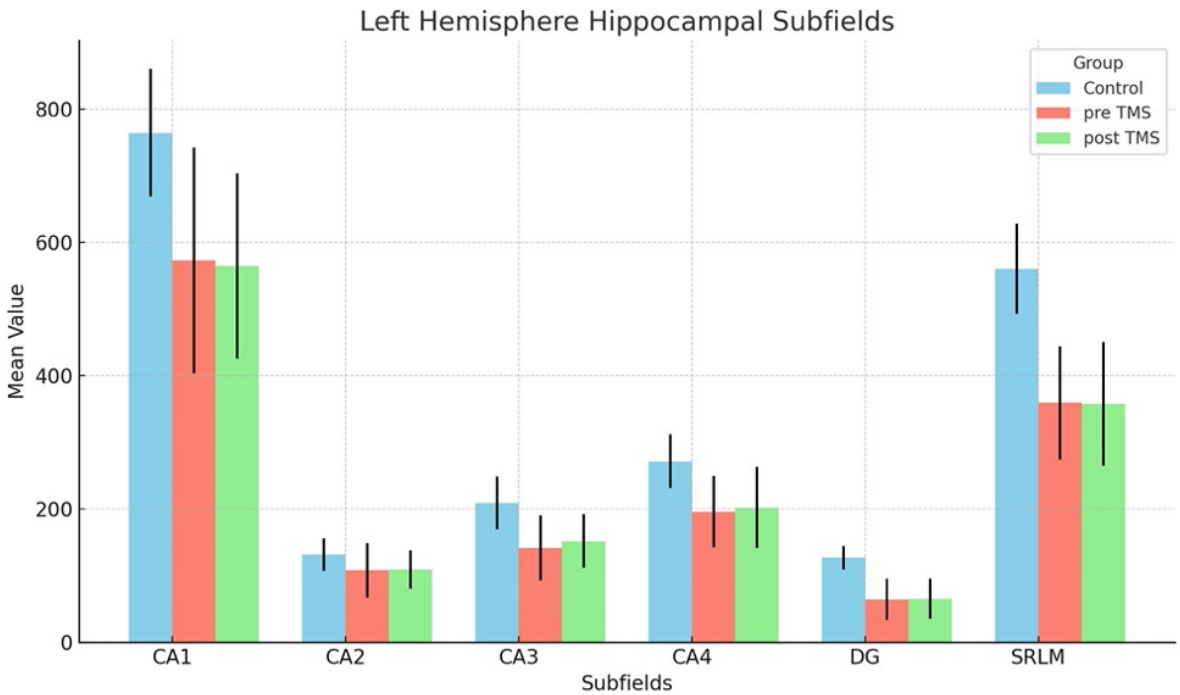

Figure 3. Mean hippocampal subfield volumes in the left hemisphere for healthy controls (blue), pre-Transcranial Magnetic Stimulation (TMS) Alzheimer's Disease (AD) patients (red) post-TMS AD patients (green). Subfields assessed include cornu ammonis 1 (CA1), CA2, CA3, CA4, dentate gyrus (DG), and stratum radiatum-lacunosum-moleculare (SRLM). Error bars represent standard deviations. This figure illustrates the volumetric differences in left hippocampal subfields across the three groups, highlighting potential changes following TMS treatment in AD patients as well as differences from the control group.

rophy appeared more pronounced, particularly in the CA3 subfield. This hemispheric difference supports the hypothesis that targeted stimulation provides some degree of structural preservation in the stimulated hemisphere, whereas the unstimulated side remains vulnerable to disease progression (10). Future research should explore the effects of bilateral stimulation to address this asymmetry and evaluate its potential to mitigate right hippocampal atrophy.

Vulnerability of specific subfields

The CA1 and SRLM subfields emerged as particularly vulnerable regions in AD. The significant reduction in CA3 volume in both hemispheres, with additional progressive atrophy observed in the right CA3 post-TMS, highlights its susceptibility to neurodegeneration. CA1 is critically involved in memory encoding and pattern separation, functions that are heavily impaired in AD (11). The pronounced decline in right CA3 volume underscores the importance of targeting this region in future interventions, potentially through bilateral or region-specific TMS protocols.

Similarly, the DG exhibited consistent atrophy in AD patients compared to HC, regardless of TMS intervention. This finding aligns with the DG's established role as a site of adult neurogenesis, which is impaired in AD (12). While no significant volumetric changes were observed post-TMS, prior studies suggest that TMS may enhance neurogenesis indirectly through increased neuroplasticity and cortical connectivity (13). Future research should evaluate whether prolonged or intensified rTMS protocols could stimulate neurogenic processes in the DG, potentially mitigating cognitive symptoms of AD.

Cortical thickness and shape metrics

Beyond hippocampal volumetry, cortical thickness, and shape analyses revealed additional structural insights into AD. Significant reductions in hippocampal thickness were observed bilaterally in AD patients compared to HC, with further thinning detected between pre-TMS and post-TMS conditions. These findings suggest that TMS may not halt cortical thinning in vulnerable subregions such as the left and right dentate

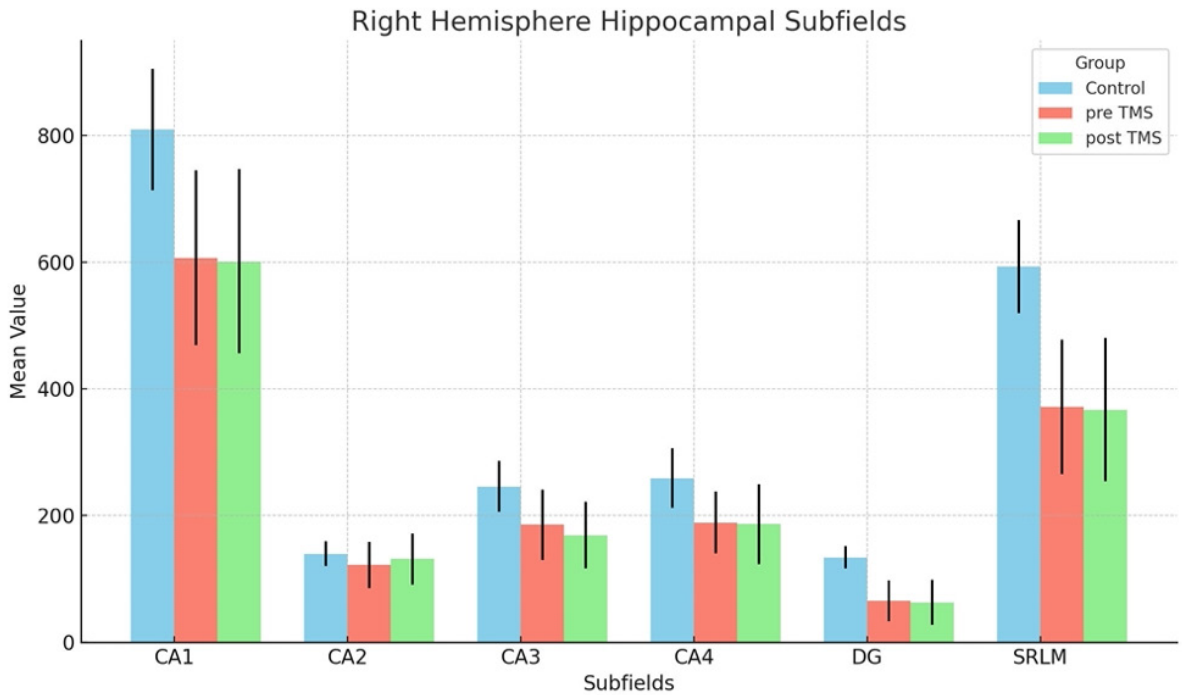

Figure 4. Mean hippocampal subfield volumes in the right hemisphere for healthy controls (blue), pre-Transcranial Magnetic Stimulation (TMS) Alzheimer's Disease (AD) patients (red) post-TMS AD patients (green). The same hippocampal subfields as in the left hemisphere are shown: Cornu ammonis 1 (CA1), CA2, CA3, CA4, dentate gyrus (DG), and stratum radiatum-lacunosum-moleculare (SRLM). Error bars represent standard deviations. This figure allows for comparison of right hippocampal subfield volumes across groups, providing insight into hemispheric differences and the effects of TMS in AD patients compared to healthy controls.

gyrus and hippocampal subfields. Cortical thinning in AD is consistent with the disease's well-documented progression, which involves synaptic loss and neuronal atrophy in regions critical for memory and learning, including the hippocampus (14).

Shape metrics, including curvature and surface area, also revealed distinct patterns of structural alterations. Notable reductions were observed in both the dentate gyrus and hippocampal surface, indicating continued degeneration in cortical and subcortical structures despite rTMS treatment. These changes highlight the complexity of structural remodeling in AD, as cortical atrophy is not limited to volumetric reductions but extends to changes in shape and connectivity (15). Such findings emphasize the need to explore whether longer or more targeted rTMS protocols could mitigate these progressive changes by promoting neuroplasticity and connectivity.

These results align with prior studies showing that cortical thickness and shape metrics provide sensitive markers of AD progression, even when volumetric recovery is absent. Importantly, changes in hippocampal thickness and shape metrics correlate strongly with cognitive decline, suggesting that these structural measures could serve as biomarkers for evaluating the efficacy of TMS and other therapeutic interventions (1,16).

Limitations

This study has several limitations that must be acknowledged. First, the relatively small sample size limits the generalizability of the findings and may reduce statistical power for detecting subtle structural changes. Larger sample sizes and multi-site studies are necessary to validate these results. The inclusion of a more diverse participant pool across multiple sites could also enhance the applicability of the findings to broader populations. Second, the absence of a sham (placebo) TMS group restricts the ability to attribute observed changes solely to TMS effects. Including a sham group in future research would provide critical controls for distinguishing between TMS-specific effects and natural variability in disease progression. The addition of alternative control conditions, such

as active or other forms of stimulation, could further strengthen causal inferences. Lastly, the short duration of the TMS protocol may have limited its potential to induce measurable volumetric changes. Extended protocols and longitudinal designs could better capture TMS's long-term structural effects. Future studies should also explore bilateral stimulation protocols, as unilateral approaches may not fully address asymmetrical patterns of hippocampal atrophy commonly observed in AD.

CONCLUSION

This study highlights the significant atrophy observed in hippocampal subfields and cortical thickness in AD patients and suggests that rTMS may modestly influence the progression of neurodegeneration. While no substantial recovery was observed, findings underscore the potential of rTMS as a neuroprotective intervention, particularly when applied bilaterally or over extended durations. Future research should focus on refining rTMS protocols to target specific hippocampal subfields, leveraging neurogenesis in regions like the DG, and addressing hemispheric asymmetry in treatment effects.

Conflict-of-interest and financial disclosure

The authors declare that they have no conflict of interest to disclose. The authors also declare that they did not receive any financial support for the study.

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