NÖRO PSİKİYATRİ ARŞİVİ
ARCHIVES OF NEUROPSYCHIATRY

BRIEF REPORT

Arch Neuropsychiatry 2024;61:189–192
https://doi.org/10.29399/npa.28420

Repetitive Transcranial Magnetic Stimulation Improves Hippocampal N-Acetlaspartate Levels and Visual Memory Scores in Alzheimer's Disease

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ABSTRACT

The latest research into the pathophysiology of Alzheimer's Disease (AD) has included several cognitive deficits related to hippocampal functioning. However, current clinical research fails to consider the full extent of the heterogeneous cognitive spectrum of AD, resulting in a lack of the specific methods required to draw definitive diagnostic and therapeutic conclusions. This also includes in-vivo metabolic measurements for tailoring the diagnostic and therapeutic regimens in humans with AD. Magnetic resonance spectroscopy and repetitive transcranial magnetic stimulation (rTMS) are two novel diagnostic and therapeutic approaches that must be modified to treat AD. In the present study, we aimed to investigate the underlying therapeutic role of rTMS in humans with AD by evaluating the in-vivo hippocampal metabolites before and after rTMS treatment. Based on the data obtained using the fMRI data in our previous study and on the references reported in the literature, in the present study, we decided to use hippocampal NAA data after rTMS stimulation and found a significant increase in NAA levels. To the best of our knowledge, no other study has evaluated the effect of rTMS on hippocampal metabolites in humans with AD.

Keywords: AD, hippocampus, MRS, NAA, rTMS

Cite this article as: Velioğlu HA, Sayman D, Hanoğlu L, Akan GE, Çankaya Ş, Yuluğ B. Repetitive Transcranial Magnetic Stimulation Improves Hippocampal N-Acetlaspartate Levels and Visual Memory Scores in Alzheimer's Disease. Arch Neuropsychiatry 2024;61:189–192.

INTRODUCTION

The latest research into the pathophysiology of the Alzheimer's disease (AD) has included several cognitive deficits related to hippocampal functioning (1). Cognitive deterioration has been reported as a heterogeneous phenomenon leading to severe ambiguity in both diagnosis and treatment. Current research methods fail to consider the full extent of the heterogeneous cognitive spectrum of AD, resulting in a lack of the specificity required to draw definitive diagnostic and therapeutic conclusions. This has suggested the importance of metabolic measurements for tailoring AD's diagnostic and therapeutic regimens. Several neuroimaging studies using Magnetic Resonance Spectroscopy (MRS) have identified abnormalities at the molecular level related to the pathology of AD (2). An important technique for understanding the functionality of neuronal and glial cells and cerebral energy metabolism in healthy and diseased brains is proton resonance of hydrogen (1 H)-MRS.

Repetitive transcranial magnetic stimulation (rTMS) is a novel method employed to modulate cortical activity in a non-invasive manner. It is commonly used to treat neuropsychiatric diseases such as depression and schizophrenia and symptoms in neurodegenerative dementias, including AD (3). The major obstacle to the use of rTMS, its inability to directly stimulate the deeply located hippocampus, has been overcome with novel stimulation approaches, such as the stimulation of specific superficial brain regions that are highly connected with the hippocampus, enabling indirect alteration of the target subcortical region, such as the hippocampus (4). One of these potential stimulation areas is the left parietal cortex, which, together with its connections to the hippocampus, is engaged in a variety of cognitive activities. In our recent study we showed significantly improved cognitive scores following parietal cortex-oriented rTMS, correlated with increased brain-derived neurotrophic factor levels and decreased oxidative

Highlights

- **The pathophysiology of Alzheimer's Disease depends on hippocampal functions.**
- **rTMS provides a new approach to the diagnosis and treatment of Alzheimer's disease.**
- **Repetitive transcranial stimulation has effects on hippocampal metabolites.**

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Received: 26.01.2023, **Accepted:** 10.04.2023, **Available Online Date:** 01.06.2024

status in blood samples from patients with AD (4). Similar positive cognitive outcomes have been replicated in human clinical trials showing improved memory performance in healthy and different disease conditions (3), although the underlying mechanisms of the pro-cognitive effect of rTMS remain largely unknown. This may be related to the complexity and interconnections within and between the metabolic pathways following brain stimulation, a topic of particular importance in recognizing the treatment framework in AD, which was not discussed in prior studies. However, a recent rat study showed that brain stimulation may induce cholinergic neurotransmission, resulting in improved cognitive outcomes, leading to relevant hypothetical questions in humans regarding whether rTMS might have a similar effect via neurometabolic pathways such as NAA levels which is known to have a role in AD pathophysiology and improving the existing cognitive state or potentially preventing further cognitive deficits (5). This question is relatively simple to answer under controllable experimental conditions. However, in living humans it is nearly impossible to collect direct metabolic information from intracellular and extracellular tissues. On that basis, we therefore set out to investigate the underlying pro-cognitive role of rTMS in humans with AD. Based on the data obtained using the fMRI data in our previous study (6) and on the references reported in the literature, in the present study, we decided to use hippocampal NAA data after rTMS stimulation. To the best of our knowledge, no other study has evaluated the effect of rTMS on hippocampal cerebral metabolites in humans with AD, and this is the first research to explore the in vivo hippocampal correlates of the pro-cognitive effects of rTMS in patients with AD.

METHODS

Subjects

Sixteen Alzheimer's patients were recruited for the MRS evaluation before and after rTMS application. However, 4 patients' MRS data were not recorded properly and excluded from the study. Only 12 patients (7 women, age 69.66±8.56) MRS data was used for our study. In addition to that two patient's choline values were not detected. An expert neurologist used the NINCDS– ADRDA criteria to make the clinical diagnosis of AD. The individuals taking part in the study had Clinical Dementia Rating Scale (CDR) scores ranging from 1–2. Acetylcholinesterase inhibitors and/or memantine were being taken by the patients. Having metallic implants, being unable to walk on one's own, being physically handicapped, having a history of alcohol or drug abuse, having mental illnesses like schizophrenia or delirium, or having epilepsy, seizures, a brain tumor, or trauma were all listed as exclusion criteria.

All patients were suitable for TMS application and MRI screening according to TMS and MRI safety-screening questionnaire (7). Our study was accepted The Ethical Committee of Istanbul Medipol University (Ethical report: 10840098– 604.01.01–E. 39987). All patients or their relatives signed the informed consent to attend our study. Patients were evaluated throughout the study period while continuing their regular therapy and receiving no modifications to their medical care.

Cognitive Evaluation

For global cognitive assessment, attention functions, general neuropsychological evaluation, language skills, memory functions, visual and perceptual functions and executive functions were evaluated with specific cognitive tests as detailed in our previous study (6) before and after TMS by the same qualified neuropsychologist who was also blind to the study hypothesis.

MRS protocol and analysis

The MRS data were taken from 3 Tesla Philips Achieva MR device (Philips Medical Systems, Best, Netherlands) before and after 2 weeks rTMS application. For the MRS imaging, 8 channel head coil was used. After patients were positioned in front of the MR device, the heads were fixed with sponge molds to prevent head movements. The MRS recording which took 7–9 minutes parameters was (TR: 2000/TE: 57 ms), ACQ voxel MPS (10/10/15 mm), FOV (RL: 230 mm, AP: 190 mm), voksel size (RL: 10 mm, AP: 10 mm), slice thickness (15 mm), VOI size (AP: 110 mm, RL: 100 mm), spectral BW (2000 Hz), configuration matrix (24 Hz).

H-MRS voxels of interest measuring 20×20×15 mm^3 were defined in a standard location in left hippocampus using a previously published method (8). We obtained the value of N-acetyl aspartate (NAA), creatine (Cr) and choline (Cho) levels in the left hippocampus via the MRS software SPectroview (Philips Healthcare) and the proportional data of NAA/Cr, NAA/Cho and Cr/Cho. All spectra were processed with SpectroView (Figure 1) as described in a previous study by Filippi et al. (9)

For statistics, Wilcoxon signed-rank test was performed to evaluate the differences between NAA, choline, creatine, NAA/Creatine, NAA/Choline and Creatine/Choline levels of pre and post-rTMS. Also, for the evaluation of the correlation between NAA levels and visual recognition scores, a correlation matrix was performed.

rTMS application parameters

We determined a stimulation coordinate for each patient using individual maps of hippocampal resting-state functional connectivity collected before the TMS therapy and we applied rTMS to these coordinates corresponding to the left lateral parietal cortex, Brodmann area 7 through the instrument of the "Brainvoyager TMS Neuronavigation" system as described in our previous study in detail (6).

RESULTS

rTMS improved baseline NAA levels (pre 1.425±0.30) to a significant degree (post 1.805±0.45, p=0.034, Wilcoxon signed-rank test) (Table 1). Among several tests applied (p>0.05, non–significant), we have found that visual memory recognition scores were significantly improved after rTMS application (pre 1.583±1.24 vs. post 2.75±1.215, p=0.014, Wilcoxon signed-rank test) (Table 1) which was also significantly correlated with the increased NAA levels (p<0.05, Pearson's r test, Table 2) Also, subjects' choline, creatin, Choline/Creatine, NAA/Choline and Creatine/Choline levels were calculated but these parameters showed no significant difference between groups. (p>0.05, Willcoxon signed-rank test) (Table 1).

DISCUSSION

In the present study, we have observed a significant increase in cognitive parameters along with improved hippocampal metabolic parameters after rTMS stimulation which was confirmed with the correlation analysis that critically improved cognitive parameters were significantly associated with increased NAA levels.

The role of cerebral metabolites in AD is largely unknown, and differences in cognitive ability are often overlooked in patients despite the association between neurochemicals and specific neuronal networks, such as dopamine levels related to attentional brain networks (10). Research to date has highlighted the importance of MRS techniques in AD, revealing inconsistent levels of choline but generally decreased NAA levels (2).

Furthermore, despite existing neurological and psychophysiological differences, there is a lack of cognitive and neurochemical data before and after rTMS application across different AD subgroups.

Figure 1. Axial T1 weighted magnetic resonance image from an AD subject illustrating the location of single voxel H-MRS voxels in the left hippocampus.

Table 1. Metabolite levels and visual recognition scores before and after the rTMS application (baseline and post– treatment metabolite levels)

	Pre TMS (n=12)			Post TMS (n=12)			Results	
Wilcoxon Test	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Statistic**	**p**
NAA	1.425	1.374	0.301	1.805	1.899	0.456	11.5	0.034
Cr	1.383	1.279	0.384	1.605	1.605	1.681	18	0.11
Cho	1.257	1.243	0.266	1.326	1.195	0.499	32	0.622
NAA/Cr	1.538	1.53	0.45	1.606	1.615	0.252	27	0.625
Cho/Cr	1.074	1.01	0.417	0.883	0.89	0.175	38.5	0.284
NAA/Cho	1.519	1.585	0.575	1.855	1.8	0.521	18	0.205
Visual Recognition	1.583	1	1.24	2.75	3	1.215	3.5	0.014

Cho: Choline; Cr: Creatine; n: Number of patients; NAA: N– acetylaspartate; SD: Standard deviation.

Table 2. The correlation between increased NAA levels and improved visual memory recognition and orientation scores

Correlation Matrix		**NAA**	**Visual Recognition**
NAA	Pearson's r	-	
	p-value	-	
Visual Recognition	Pearson's r	0.439*	-
	p-value	0.032	-

NAA: N-acetylaspartate.

A beneficial activity-dependent neurochemical effect was observed in hippocampus. The findings revealed significantly altered NAA levels, suggesting the possibility of an accelerated neuronal healing process. Since many of the synapses in the hippocampus are cholinergic, and mitochondrial NAA synthesis is highly dependent on acetyl-CoA even disturbed cortical cholinergic innervation leads to a reduction of NAA synthesis (11) it was not surprising to observe that hippocampal-oriented rTMS improved both spatial and episodic memory in patients with AD in our study. This also explains the low NAA levels in patients with AD with cholinergic deficits that are restored by means of cholinergic treatment, leading to improved cognition. Besides suggesting the pro-cognitive role of rTMS in depression (12; 13), and traumatic brain injury (14), our present findings fit well with recent data showing beneficial changes in left dorsolateral prefrontal cortex (DLPFC) NAA levels after rTMS in patients with AD (15).

Given creatine's pro-energetic and neuroprotective role in a variety of neurological disorders (16), an increase in brain creatine levels following rTMS application was not surprising (Table 1). Our findings also fit well with a recent AD study showing decreased creatin levels in both hippocampi (8).

Despite the valuable metabolic information yielded by the current study, the number of subjects in each group was relatively small, and a larger sample would be desirable to obtain more accurate results. Our discovery that rTMS can generate detectable and long-lasting biochemical changes in the hippocampus linked with better cognitive metrics in AD is a significant strength of this study. Our data regarding increased NAA signals may also indicate that rTMS can exert considerable effects on hippocampal neuronal integrity and metabolism, a phenomenon of potential neuroprotective value. Combining rTMS and modern neuroimaging tools with bioenergetic markers may yield further insights into the mechanisms underlying the degeneration occurring in AD, possibly due to energetic deficiencies.

Ethics Committee Approval: Our study was accepted The Ethical Committee of Istanbul Medipol University (Ethical report: 10840098– 604.01.01–E. 39987).

Informed Consent: All patients or their relatives signed the informed consent to attend our study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept- BY, HAV; Design- BY, LH; Supervision- ŞÇ; Resource- HAV; Materials- HAV; Data Collection and/or Processing- LH, GEA; Analysis and/or Interpretation- BY, LH; Literature Search- BY DS; Writing-DS, HAV ; Critical Reviews- BY, ŞÇ.

Conflict of Interest: The authors declared that there is no conflict of interest.

Financial Disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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