Short and Long-term Effects of rTMS Treatment on Alzheimer's Disease at Different Stages: A Pilot Study

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ABSTRACT: Repetitive transcranial magnetic stimulation (rTMS) uses a magnetic coil to induce an electric field in brain tissue. As a pilot study, we investigated the effect of rTMS treatment on 10 volunteers with Alzheimer's disease (AD) in a two-stage study. The first stage consisted of a double-blind crossover study with real and sham treatments. Each treatment block consisted of 13 sessions over 4 weeks. During each session, 2000 TMS pulses at 90%–100% of resting motor threshold were applied to dorsolateral prefrontal cortex bilaterally, and the patients were kept cognitively active by object/action naming during the treatment. The second stage was an open-label study, in which the same treatments were performed in 2-week blocks (10 sessions) approximately every 3 months as follow-up treatments on six of the volunteers, who completed the first stage of the study. Primary outcome measures were the Montreal Cognitive Assessment (MOCA) and the Alzheimer's Disease Assessment Scale-cognitive subscale. The secondary outcome measures were the Revised Memory and Behavior Checklist as well as our team's custom-designed cognitive assessments. The results showed a noticeably stronger improvement on all assessments during the real treatment as compared to the sham treatment. The changes in MOCA scores as well as our designed cognitive assessment were found to be statistically significant, with particularly strong results in the six volunteers who were in the early stages of the disease. The long-term trends observed in the second stage of the study also showed generally less decline than would be expected for their condition. It appears that rTMS can be an effective tool for improving the cognitive abilities of patients with early to moderate stages of AD. However, the positive effects of rTMS may persist for only up to a few weeks. Specific skills being practiced during rTMS treatment may retain their improvement for longer periods.

KEYWORDS: Alzheimer's disease, treatment, transcranial magnetic stimulation, cognitive assessments, MoCA, ADAS-Cog

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Introduction

Dementia, and specifically Alzheimer's disease (AD), is a growing problem in our society as life expectancy increases. Current treatments for AD are unable to cure or halt the progress of the disease, and have only mixed results in alleviating the symptoms. The most commonly used medication, Donepezil, shows some benefit for 20%–60% of patients,[1] but a substantial and marked benefit for only 2.3%.[2] However, a long-term study showed no significant benefit compared to placebo for improving daily living functions of Alzheimer's patients,[3] and many patients discontinue it due to severe side effects.[1,2] This study investigates a new protocol on the use of repetitive transcranial magnetic stimulation (rTMS) as a potential treatment for AD. rTMS is a technique that has been successfully used to treat the symptoms of various neurological and psychiatric disorders, including depression, schizophrenia, and Parkinson's disease.[4] It is a noninvasive, nonpharmacological technique that is quick to administer and relatively easy for patients to tolerate, with no lasting side effects.

rTMS involves applying a rapidly changing magnetic field to the outer surface of the brain.[5] This magnetic field is produced by running a strong electrical current through a conducting wire in a circular or figure-of-eight shaped coil. The rapidly varying magnetic field produced by this coil when it is positioned over the subject's skull is able to induce electrical fields in the conductive brain tissue, which results in ion movements and can depolarize or hyperpolarize neurons. Single pulses of TMS to the motor cortex are able to elicit muscle activity, and repetitive pulses (rTMS) have been shown[6] to affect the excitability of the stimulated region, depending on the frequency of the pulses. Specifically, low-frequency pulses (around 1 Hz) seem to decrease cortical excitability, while high-frequency pulses (10–20 Hz) seem to increase cortical excitability in most subjects.[6,7] The mechanism by which this happens is thought to be long-term potentiation (LTP)/long-term depression (LTD) due to the similarity of the effects of rTMS to the features of LTP/LTD, although direct evidence of a causal link for LTP/LTD as a result of rTMS is lacking.[8]

AD is characterized by neuronal death and increased characteristic markers such as amyloid beta plaques and neurofibrillary tangles. In order to counteract the effects of neuronal

death, and the particular susceptibility of cholinergic cells in AD, most pharmacological treatments rely on acetylcholinesterase inhibitors to increase the excitability of cells that respond to acetylcholine. Thus, the goal of current treatments is to increase the excitability and activity of remaining cells in order to counteract the decline in brain function. Other proposed treatments for AD, such as mental exercises, also aim to increase the level of activity in the brain. Since rTMS has been shown to be able to both stimulate activity and to increase excitability of neural tissue, we hypothesize that it will have a beneficial effect on patients for the same reasons as acetylcholinesterase inhibitors and mental exercises are useful.

A few groups have already investigated the effect of rTMS on specific aspects of cognitive functioning in AD patients in small samples.[9–15] Reviews of these studies can be found in Refs 16–19. Some promising results include an improvement in object- and action-naming tasks during application of rTMS;[9,10] an improvement in sentence comprehension for up to 8 weeks after treatment;[11] and improvements in various cognitive measures over periods of up to 3 months.[12] A promising recent study showed that 18 weeks of rTMS application plus cognitive training provided during the same session of treatment (6 weeks every day with maintenance treatments of 2 days/week for 12 more months) provided significant cognitive benefits measured by Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) assessment compared to that of placebo group.[14] Another recent study showed some improvement in certain language tasks, which was maintained for 4 weeks post treatment.[15]

The most important parameters of an rTMS treatment are the choices of frequency and which brain region should be stimulated. All previous studies of rTMS treatment applied to Alzheimer's have used high-frequency (HF: 10–20 Hz) stimulation to the dorsolateral prefrontal cortex (DLPFC) brain region (bilaterally) in order to increase cortical excitability. DLPFC plays an important role in executive function of the brain, such as decision making; it is involved with coordinating activities of the rest of the brain, including storage and retrieval of information, and therefore has a role in working memory. Dementia is characterized by problems in working memory and adaptive decision making. Thus, it is concluded that the DLPFC is affected by dementia.[20] In the Alzheimer's brain, there is profound impairment of metabolic interactions with astrocytes due to an abnormal glutamate–glutamine (Glx) cycle.[21] Application of HF-rTMS to the left DLPFC area has been shown to increase Glx levels and restore the Glx cycle to normal;[22] it also increases cerebral blood flow and glucose metabolism in stimulated and remote brain regions[23] and reduces intracortical inhibition.[24] On the other hand, HF-rTMS application to the right DLPFC area has been shown to alleviate anxiety symptoms,[25] which are shown to be significantly higher in Alzheimer's patients at mild to moderate stages.[26,27] Enhanced synaptic plasticity has been suggested as a potential mechanism for the effect of HF-rTMS.[8] For the above reasons, similar to all other relevant research, we chose to apply HF-rTMS bilaterally to the DLPFC.

This paper presents the results of our two-stage study investigating the effect of HF-rTMS treatment on AD patients applied bilaterally to the DLPFC. In the first stage, patients were treated for 13 sessions in 4 weeks, and the duration of any positive effects was investigated. In addition, we investigated whether additional follow-up treatment every 3 months would improve or stabilize the patient's cognitive state.

Methods

Patients. Eleven volunteer patients (seven women) in the age range 57–87 years participated in this study. Out of the 11 patients, 10 finished the entire protocol of the study, and one woman discontinued because her method of transportation to treatment sessions was no longer available to her. All patients and also their primary caregiver signed an informed consent form approved by the University of Manitoba Biomedical Research Ethics Board (BREB) prior to the experiments. The University of Manitoba BREB approved the research, which was conducted in accordance with the Declaration of Helsinki.

The inclusion criteria for our study were to meet all the following conditions: 1) a diagnosis of probable AD from their neuropsychiatrist or neurologist; 2) an initial Montreal Cognitive Assessment (MOCA) score between 5 and 26 (out of 30); 3) no history of seizures and no metal in their body (safety reasons related to the use of rTMS); 4) between 40 and 90 years of age; 5) taking a stable dose of any medications used to treat AD for at least 3 months prior to the study and have no plans to change medication for the duration of the study; and 6) being English speaking, and be able to arrange their own transportation to the treatment site accompanied by the help of their caregivers. Volunteers were deemed ineligible for the study if they had a diagnosis of any other type of dementia, a diagnosis of any other neurological condition, any major injury or surgery to the head, or moderate-to-severe depression.

All volunteers were tested for depression using the Montgomery–Åsberg Depression Rating Scale (MADRS) and disqualified from the study if this test indicated moderate or severe depression (a score of 20 or higher). Only one of the selected patients (patient P2) was determined to have mild depression using the MADRS scale; all others were found to have no depressive symptoms. This was done because it is possible that rTMS would treat the symptoms of depression and confound the investigation into the effects of rTMS on the symptoms of AD.[18]

Volunteers were monitored during treatment for symptoms of discomfort or seizure. A few volunteers described minor discomfort, which occasionally made us to pause treatment for few minutes. Minor headaches were occasionally described, but not considered to be an issue by the volunteers or their caregivers. No symptoms of seizure were observed.

Study design. The study design included a double-blind, placebo-controlled Stage 1 and an open-label, follow-up Stage 2 of HF-rTMS treatment. In Stage 1, to have a measure for

placebo, we made it to be a crossover design due to lack of resources and the small number of recruited patients at the time. The patients were randomly assigned to two groups: one group (S-R) receiving sham treatment and then real treatment, and the second group (R-S) receiving real treatment and then sham treatment. Out of the 10 patients who finished the study, 4 were in S-R group and 6 in the R-S group. As for the sham treatment, a 2-cm-thick wooden block was inserted between the coil and the patient; this attenuated the strength of the induced electrical field in the brain tissue well below the threshold required to stimulate neurons without affecting the sound or tactile experience of the treatment. None of the patients noticed any changes between sham and real treatment.

Treatment protocol. In this study, we had a 4-week block of double-blind treatment (Stage 1) followed by 2 weeks of open-label maintenance treatments repeated approximately every 3 months (Stage 2).

In order to design and test an optimal rTMS protocol for the treatment of AD, we reviewed various studies investigating the cognitive benefits of rTMS on AD.[18] Most of the beneficial effects observed so far have resulted from stimulating both the left and right DLPFC at a frequency of 20 Hz and an intensity of 90%–100% of the pulse strength required to evoke a visible motor response over the motor cortex.[18] One study[11] indicated that 4 weeks of treatment did not significantly improve results over a 2-week treatment. Thus, we selected a treatment schedule of five visits per week for 2 weeks with three additional maintenance visits for a further 2 weeks (13 treatment sessions in total). To investigate the placebo effect, given the small sample size, we designed the study as a crossover study with a two-block treatment protocol, in which one block involved treatment with a real coil and the other with a sham coil. The patients, their family members, and the trained cognitive evaluators performing the ADAS-cog assessments were unaware of the patient's assignment to real-then-sham or sham-then-real treatment order blocks. There was a 4-week washout period between the two blocks of treatment. Table 1 gives a summary of the treatment schedule.

Following completion of the first stage of the study, all patients, regardless of their initial group assignment, were invited to continue with an open-label long-term follow-up study. Six of the patients selected to do so, and they received 2 weeks (5 days/week) of real rTMS treatments approximately every 3 months. However, due to scheduling issues, the actual time interval between follow-up visits varied from a minimum of 2 months to a maximum of 7 months. The stimulation parameters of the follow-up treatment were identical to those of the first stage of the study. Partial assessments were done at the beginning of every week, and a detailed assessment was done on the Monday following the final treatment of the block.

In both stages of the study, the magnitude of the rTMS pulses was set to between 90% and 100% of the resting motor threshold (RMT) intensity, the intensity required to produce a visible movement when single pulses were applied to the motor cortex. The RMT of patients were measured by applying single pulses over the C3 area of the motor cortex (from a 10–20 EEG System) on each session before applying the rTMS. The position of the coil was adjusted slightly until three consecutive finger movements were observed, and then the intensity was decreased in 5% steps until no finger motion could be seen. The lowest intensity at which finger motion could be evoked consistently was considered to be the RMT.

During each treatment session, rTMS was applied over both the left and right DLPFC, which was located using measurements from fixed anatomical positions.[28] Pulses were given in 2-second bursts at 20 Hz (40 pulses per burst) with 5-second inter-train intervals between the bursts. Fifty bursts

Table 1. Treatment and assessment schedule—first stage.

	WEEK	ASSESSMENTS (MONDAYS)	NO. OF TREATMENTS
First treatment block (real or sham)	0 (Baseline)	ADAS-cog, RMBC, spatial awareness, word–image association, associative memory, MOCA	5–Monday to Friday
	1	MOCA	5–Monday to Friday
	2	MOCA	2–Monday and Wednesday
	3	MOCA	1–Monday
	4	ADAS-cog, RMBC, spatial awareness, word–image association, associative memory, MOCA	None
Washout	Minimum 4 weeks	None	None
Second treatment block (real or sham)	8 (Baseline)	ADAS-cog, RMBC, spatial awareness, word–image association, associative memory, MOCA	5–Monday to Friday
	9	MOCA	5–Monday to Friday
	10	MOCA	2–Monday and Wednesday
	11	MOCA	1–Monday
	12	ADAS-cog, RMBC, spatial awareness, word–image association, associative memory, MOCA	None

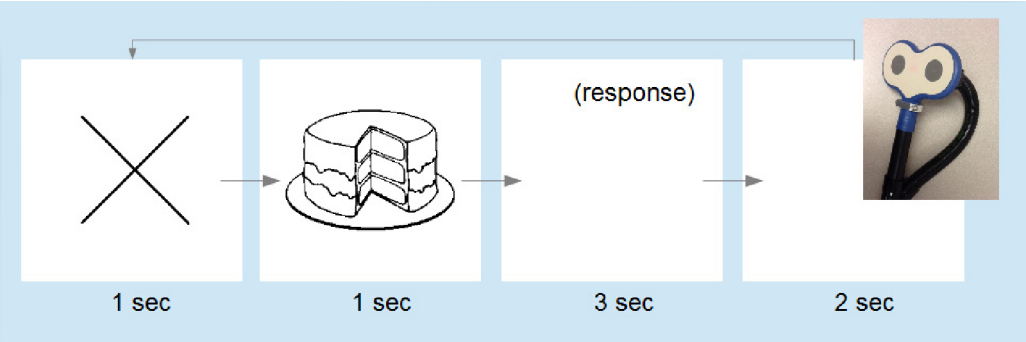

Figure 1. Illustration of the timing of images presented to the patients.

were applied to each side of the brain, for a total of 2000 pulses to each of the right and left sides per session.

During the 5-second delay between the bursts, an image was presented for 1 second on a projection screen in front of the patient (Fig. 1). The patient was asked to name the object or action depicted in the image as quickly as possible. The purpose of this task was to keep the patient cognitively active while rTMS was being applied. Patient performance on this task was not evaluated.

Assessments. In the first stage of the study, patients were evaluated before the first treatment session (baseline) and 4 weeks later after the final treatment session (Table 1). Since all patients were given two treatment blocks, one real and one sham, separate baseline and final assessment were done for each treatment block. In the second stage of the study, full assessments were done on the Monday following the final treatment day (Table 2).

Various forms of assessment were administered. The ADAS-cog test[29] was assessed by a trained clinical psychologist, who was blind to the real/sham group assignment. This test evaluates the patient's memory, language, attention, and other cognitive abilities. A different form of the ADAS-cog, with different entries for word list recall and recognition, was administered at each test session to avoid practice effect. The Revised Memory and Behavior Checklist (RMBC)[30] was given to caregivers, who were also blind to real/sham group assignment, to assess how the patient's behavior was affecting their daily lives. The MOCA test[31] was administered to patients on the first visit of every week during treatment, during both stages of the study. This assessment measures the visual, language, memory, and cognitive skills. It should be noted that unlike the ADAS-cog assessor, the MOCA assessor was not able to be blind to the real/sham group assignment. All patients were also evaluated using two of our designed online brain exercises[32] every 4 weeks during the first stage of the study. These exercises included associative memory tasks and word/image association. It should be mentioned that all patients were encouraged to use those brain exercises at home; however, only two of them (P1 and P3) did so consistently. Tables 1 and 2 give a summary of the protocol of rTMS and cognitive assessments.

Table 2. Treatment and assessment schedule—second stage.

WEEK	ASSESSMENTS (MONDAYS)	NO. OF TREATMENTS
0	MOCA	5–Monday to Friday
1	MOCA	5–Monday to Friday
2	ADAS-cog, MOCA	None
2–7 month intervals between blocks	None	None

Statistical analysis. The first stage of the study was analyzed using repeated measures two-factor analysis of variance (ANOVA), with time as the repeated factor and group assignment as the between-subjects factor. The data for the S-R group was reordered so that the real and sham treatments lined up with the corresponding treatments in the R-S group. Tests were done to determine whether there were significant differences between the two groups, which would indicate that the order of the treatment had a significant effect (S-R vs R-S). Tests were also done to determine whether there were significant effects among the real and sham treatments weeks. The Huynh–Feldt correction for sphericity was used. All ANOVA calculations were done using the SPSS 14.0 package. Following successful ANOVA results, two-tailed paired *t*-tests were used to compare each week with the corresponding sham week and determine which showed significant improvement with the real treatment. In case of missing values, mean imputation was used. All results passed a test for normality using skewness and kurtosis.[33] In all instances, a *P*-value of 0.05 or less was considered significant.

The second stage of the study was analyzed by calculating a linear regression slope from all the data available from the subject (from both stages), and comparing the result to the expected age-adjusted decline rate of Alzheimer's patients, which was derived from Ref. 34. No formal statistical tests were done on the second stage due to the low number of data points ($N = 6$).

Results

The ANOVA analysis of the ADAS-cog data from the first stage of the study showed a lack of significance for the

between-group effects and also no significant effect due to treatment type or week. The changes of ADAS-cog scores from baseline are shown in Table 3. Note that the ADAS-cog assessment scale measures errors and RMBC measures the distress level of the caregiver of the patient; thus, a decrease in score of either of ADAS-cog and RMBC indicates an improvement in cognitive ability. The missing data were due to scheduling issues (unavailability of the patients on the scheduled day due to nonrelated illness), which certainly contributed to our nonsignificant results. As can be seen in Table 3, on average ADAS-cog and RMBC scores show more improvement after the real treatment compared to those of the sham treatment, but these changes were not statistically significant. Nevertheless, the observed power of ADAS-Cog test was only 38%, which indicates there is a need for many more study subjects to have reliable statistical results.

For those patients who were able to complete mental exercises on a computer, their ability to perform these tasks was evaluated. Two tasks were presented: 1) an associative memory problem that involved learning associations between shapes and animals, and 2) a word–image association task that involved remembering sequences of words and responding with the correct sequence of corresponding images. For a description of these tasks, see Ref. 32. The change in score for these methods of evaluation before and after treatment is also given in Table 3. For both these tasks, an increase in score indicates an improvement in the ability. For both tasks, a greater average improvement was seen following real rTMS as compared to sham, but these differences were only statistically significant for the word–image association task ($P = 0.156$ for associative memory and $P = 0.040$ for word–image association).

In contrast to other methods of evaluation, which were performed only before and after each treatment, the MOCA assessment was done every week. This allowed a comparison of the cognitive ability at various points during and after the treatment. The ANOVA analysis of the MOCA data showed no significant effect due to treatment order (S-R vs R-S), which confirms that the washout period of four weeks was adequate for our purpose ($P = 0.897$). However, there was a significant effect due to week ($P = 0.019$), with the measurements on weeks 2 and 3 of the real treatment showing strong differences when compared to baseline ($P = 0.021$ and 0.017, respectively). The observed power of the ANOVA week effect test was 90%, and the observed power for the comparisons to baseline was 71% for week 2 and 74% for week 3. In contrast, none of the sham weeks showed any significant difference from baseline.

In order to further analyze these results, the baseline value at the beginning of each treatment session was subtracted, and all real treatments and all sham treatments were compared regardless of real-then-sham or sham-then-real order (Fig. 2). These results showed a significant difference between real and sham treatments using paired *t*-tests on week 2 ($P = 0.0132$). Data of the patients with missing values were dropped from the paired *t*-tests. This happened for two patients in week 1, four patients in week 2, and two patients in week 4.

Based on the ADAS-cog scores of our study participants, they were clearly in two different stages of AD (Fig. 3). Six patients had ADAS-cog scores under 25 (early stage) and four had ADAS-cog scores over 30 (advanced stage). Thus, in order to investigate the difference in treatment between different stages of the disease, the results were compared and analyzed for patients in the early and advanced stages separately.

When the "early stage" and "advanced stage" patients were separated in the analysis, it became clear that the improvement during real treatments was greater for the "early stage" group than the "advanced stage" group. This indicates a difference

Table 3. Results of ADAS-cog, RMBC, Associative Memory, and Word-Image Association (Changes from Baseline).

PATIENT	ADAS-cog CHANGE		RMBC CHANGE		ASSOCIATIVE MEMORY		WORD–IMAGE ASSOC.*	
	REAL	SHAM	REAL	SHAM	REAL	SHAM	REAL	SHAM
P1	–1	–2	–7	–7	–0.07	0.00	–3	–6
P2	–1	–1	–5	–9	0.80	–0.40	34	5
P3	0	3	–3	0	0.60	–0.40	35	9
P4	NA	–2	NA	–13	0.00	–0.18	19	–7
P5	NA	–3	–3	–2	NA	NA	NA	0
P6	NA	3	–5	–10	0.67	NA	11	NA
P7	–6	–8	–16	7	NA	NA	NA	NA
P8	NA	2	–17	6	NA	NA	NA	NA
P9	–6	3	15	–15	NA	0.00	NA	15
P10	–10	–6	–11	NA	0.00	0.00	NA	–16
Average	–4.00	–1.10	–5.78	–4.78	0.33	–0.16	19.20	0.00
Std Err	1.61	1.23	3.13	2.66	0.16	0.08	7.17	4.00

Note: *Significant difference using paired *t*-tests.

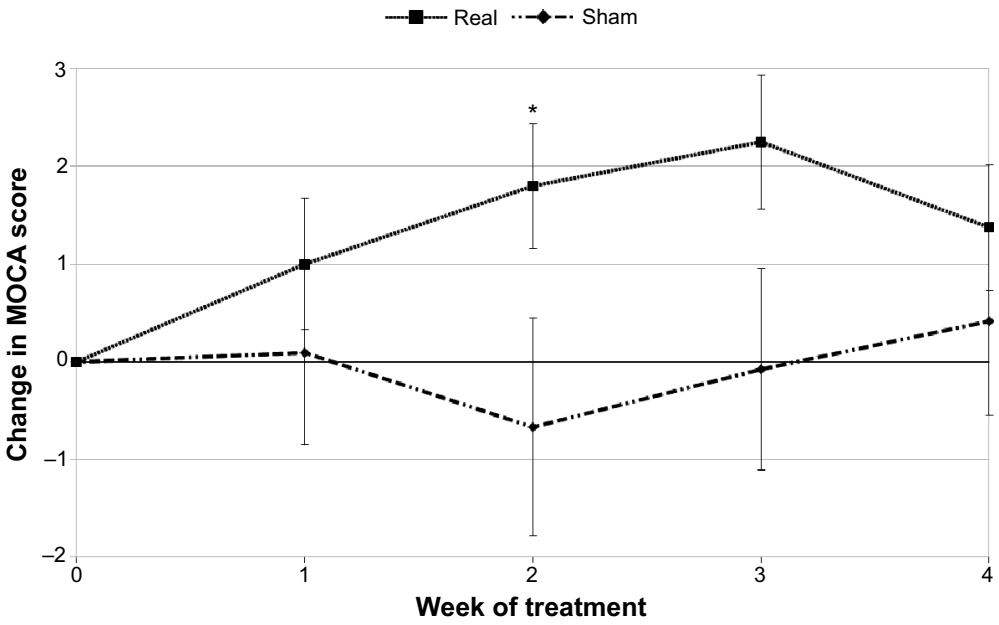

Figure 2. Change in MOCA scores averaged among the patients. The dotted and dashed lines show the scores of real and sham treatments, respectively. The bars show standard errors. Stars indicate significant differences using paired *t*-tests.

in the response to the treatment between these two groups. When only the "early stage" patients' data were analyzed, the difference between the real and sham treatments was much stronger (Fig. 4). For the "early stage" patients, the improvement of the real treatment as compared to sham was also significant using paired *t*-tests in week 3 ($P = 0.0057$).

The second stage of the study involved long term-measurements of six patients; they received 2 weeks of treatment every 2–7 months, with MOCA assessments every Monday and an ADAS-Cog assessment following treatment (Table 2). These patients have been participating for a minimum of 10 months and a maximum of 19 months. The assessment results of these patients on the second follow-up stage were analyzed by fitting regression lines to patients' scores over time. It should be noted that the volunteers who continued to this stage of this study were those who were most satisfied with the treatment in the first stage of the study, so they cannot be considered a random sample. As there was no control group for this part of the study, no formal statistical tests are presented; however, we derived decline rates of AD patients from Ref. 34 as a point of comparison. Both MOCA and ADAS-cog scores were collected for this study, and MOCA scores were converted to Mini-Mental State Examination (MMSE, a similar and simpler version of the MOCA test) equivalents using the method provided in Ref. 35 so that they could be compared to the published long-term decline rates

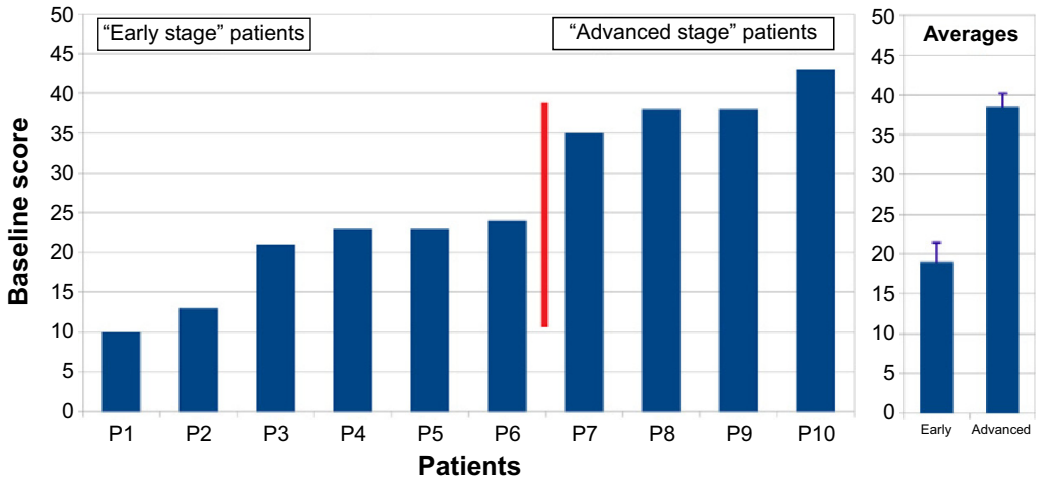

Figure 3. Baseline ADAS-cog scores. Two distinct levels of ability were identified in our patients: Six "early stage" patients and four "advanced stage" patients. "Early stage" patients performed significantly better on the ADAS-cog assessment at baseline.

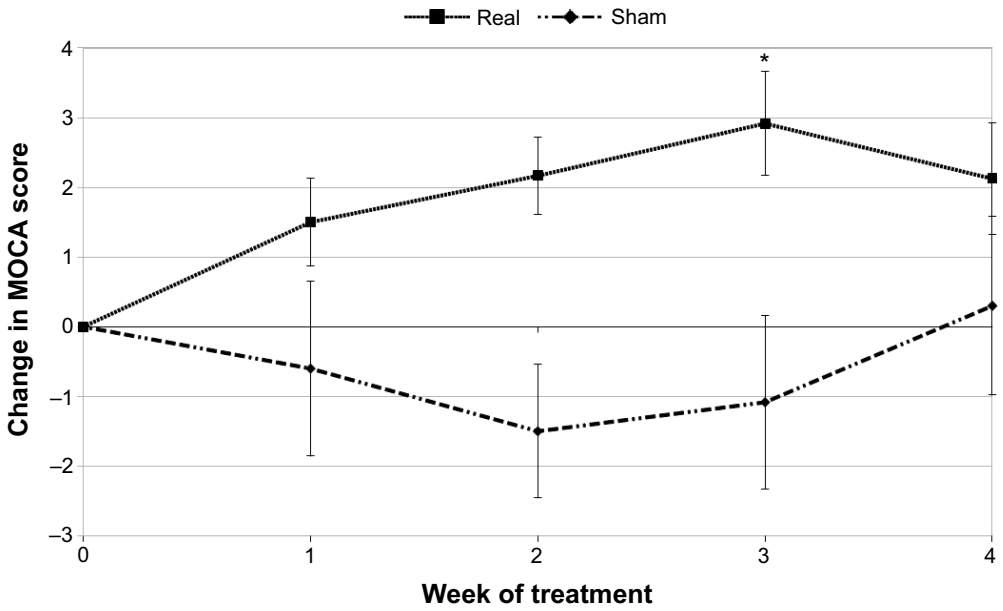

Figure 4. Change in MOCA scores averaged among the "early stage" patients (six patients). The dotted and dashed lines show the scores of real and sham treatments, respectively. The bars show standard errors. Stars indicate significant differences using paired *t*-tests.

for AD in Ref. 34. These results are summarized in Table 4. Note that all decline rates of our study patients were better than the expected rate, with the exception of the ADAS-cog scores for patients P5 and P8.

Discussion

The results of the MOCA assessments are quite compelling, particularly for the "early stage" patients. In week 2 (full group) and 3 ("early stage") of treatment, there was a strong and statistically significant difference between real and sham responses to the MOCA test. While no other groups have used this particular measure for tracking change in cognition, this is consistent with improvements in language abilities[11,15] and general cognitive abilities[12,14] found in other studies. It should be noted that, in previous studies, these cognitive improvements have been found to persist for multiple weeks after treatment[12] or during lengthy periods of biweekly maintenance treatments,[14] while our results were only significant for a short time following the 2 weeks of intense treatment. Also, studies of rTMS in Parkinson's disease showed improvements that lasted for at least 1 month.[36] While our results at the 4-week assessment were not strong enough to be statistically significant, there was still a noticeable improvement in all assessed values at this stage, which may indicate that with more data a continuing improvement would be seen.

Table 4. Annual decline rates, measured using regression fits to observed data (MOCA converted to MMSE scores). Expected values calculated based on patient's age using data from Ref. 34.

SUBJECT	AGE	ADAS-cog		MMSE (CONVERTED)	
		MEASURED	EXPECTED	MEASURED	EXPECTED
P1	79	2.71	3.74	–0.06	–1.68
P3	86	–0.99	2.66	1.50	–0.65
P4	69	2.05	5.29	–0.09	–3.15
P5	78	3.95	3.90	0.15	–1.82
P8	57	7.72	7.15	–2.41	–4.91
P10	62	3.72	6.38	–1.29	–4.18

One question raised by these results is why a significant effect was seen in the MOCA scores but not as much in the ADAS-cog or RMBC scores. It is important to note that a test like ADAS-cog also depends on the mood of the patient. It is quite possible (as indeed it was the case for three patients in this study) that on the day of ADAS-cog assessment the patients were not in their best mood. It is also notable that the positive effects observed for MOCA scores had largely disappeared by the final week of treatment, which was when the ADAS-cog and RMBC scores were assessed. This suggests that general cognitive skills are improved during treatment, but the effect may not last longer than a week or two. However, as discussed, this would not be consistent with previous studies documenting relatively long-lasting cognitive benefits. This could be explained by the fact that one of the previous studies had used the MMSE rather than the ADAS-cog for assessment.[12] Also, in that study there may have been a confounding effect due to some of the volunteers suffering from depression, which can also be treated using rTMS, and this may explain some of their positive results.[12] Another study that did use the ADAS-cog for assessment used rTMS along with cognitive training at the site and also had a much longer treatment period (6 weeks).[14]

It is worth noting that our patients, particularly those at early-to-moderate stages, and their primary caregivers (their spouses) were expressing positive and significant improvement

during the course of treatment to the extent that they requested the study to be repeated and continued. The fact that this subjective but very positive feedback is not reflected as much on the ADAS-cog and RMBC measures may pose a question on the adequacy of these standard questionnaires as outcome measures. Most of our patients showed a great reluctance to undergo the ADAS-cog assessments, perhaps because assessment was lengthy and the types of questions were making them tired or frustrated; thus they might have performed poorer than their true capability. Also, according to the spouses, the questions of RMBC questionnaire do not allow them to reflect the changes that they had observed in their patients. Thus, there is probably a need to either develop new outcome measures or modify the current standard ones by reassessing their adequacy for the Alzheimer population.

One may raise the doubt that the difference between the real and sham MOCA improvements was due to practice effects. Since we performed the MOCA test weekly, it is possible that even patients with memory problems such as those with Alzheimer's would learn to perform better over time. However, we performed MOCA tests in the same manner for both sham and real treatments; thus, one may speculate that this learning due to practice effect was somehow facilitated by real rTMS treatment. Nevertheless, even with this plausible scenario, the fact that Alzheimer patients learned and remembered a task is a positive outcome. For reference, the average test–retest improvement in MOCA scores was measured to be 0.9 points over an average period of 35 days.[31] In comparison, our peak difference in MOCA scores between real and sham treatments occurred in the "early stage" group on week 3 of treatment, and was measured to be 4.0 points. This conclusion would also be supported by the significantly greater improvement during real treatment as compared to sham of the word–image association task. Since two of the six patients were practicing this task at home, it is plausible that this difference is a result of rTMS having a facilitating effect on this practice. This conclusion is also supported by the results of a recent study by Rabey et al,[14] which concludes that, when rTMS is applied together with cognitive training, the cognitive benefits are greater than when using rTMS or cognitive training alone.

The results of the second stage of our study were quite promising, and suggest that, in addition to the short-term benefits observed in the first stage, rTMS may also slow the progression of the disease over time. Possible mechanisms for this effect are neurogenesis or anti-apoptotic effects. Neurogenesis has been demonstrated in rats that were exposed to HF-rTMS,[37] and anti-apoptotic effects were seen when HF-rTMS was applied to a rat model of ischemic stroke.[38] Although we were not able to include a control group at Stage 2 (the open-label follow-up treatments), the fact that all six of our patients had much slower decline rates on the MOCA measurement than expected for their age is quite encouraging. It should be noted that two of the six patients actually improved over time on their MOCA assessments. Additionally, four of the six patients did better than expected on the ADAS-cog assessments.

Application of rTMS over the DLPFC likely activates the basal forebrain cholinergic complex (BFCC). The BFCC projects over most of the cortex, and also provides connectivity via GABAergic inputs to the midbrain regions. As a consequence, a release from inhibition may allow for increased metabolism in these midbrain regions, which are known as major sources of cholinergic, serotonergic, and norepinephrinergic inputs to many regions of the brain. This may provide a pathway for therapeutic intervention.

Overall, the results of this study support a growing pool of evidence that rTMS can be used as a treatment to mitigate some of the degenerative effects of AD. It appears that rTMS may be more effective for patients in the early stages of the disease. While the general cognitive benefits were not shown to persist for longer than a few weeks after the cessation of a treatment schedule in this study, it is also possible that rTMS has a facilitating effect on the training of tasks being practiced during a period of regular rTMS treatment. Given that the positive effect of treatment lasts only a few weeks, we suggest repeating the rTMS treatment every 2 or 3 months for 2 weeks every day. Further research is needed to evaluate the clinical significance of observed cognitive changes. Although rTMS requires specialized equipment, it is simple to administer and noninvasive. With more research, it could become a useful tool, along with mental exercises and pharmacological interventions, for improving the lives of people who suffer from AD.

Author Contributions

Conceived and designed the experiments: ZM, GR. Analyzed the data: GR. Wrote the first draft of the manuscript: GR. Contributed to the writing of the manuscript: GR, ZM, BL. Agree with manuscript results and conclusions: GR, ZM, BL. Jointly developed the structure and arguments for the paper: GR, ZM, BL. Made critical revisions and approved final version: GR, ZM. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Burns A, Yeates A, Akintade L, et al. Defining treatment response to donepezil in Alzheimer's disease. *Drugs Aging*. 2008;25(8):707–714.
2. Lanctôt KL, Herrmann N, Yau KK, et al. Efficacy and safety of cholinesterase inhibitors in Alzheimer's disease: a meta-analysis. *Can Med Assoc J*. 2003;169(6): 557–564.
3. Courtney C, Farrell D, Gray R, et al; AD2000 Collaborative Group. Long-term donepezil treatment in 565 patients with Alzheimer's disease (AD2000): randomised double-blind trial. *Lancet*. 2004;363(9427):2105–2115.
4. Guse B, Falkai P, Wobrock T. Cognitive effects of high-frequency repetitive transcranial magnetic stimulation: a systematic review. *J Neural Transm*. 2010;117: 105–122.
5. Barker AT. An introduction to the basic principles of magnetic nerve stimulation. *J Clin Neurophysiol*. 1991;8:26–37.
6. Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A. Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. *Exp Brain Res*. 2000;133:425–430.
7. Daskalakis ZJ, Christensen BK, Fitzgerald PB, Chen R. Transcranial magnetic stimulation: a new investigational and treatment tool in psychiatry. *J Neuropsychiatry Clin Neurosci*. 2002;14:406–415.

8. Hoogendam JM, Ramakers GMJ, Di Lazzaro V. Physiology of repetitive transcranial magnetic stimulation of the human brain. *Brain Stimulat*. 2010;3:95–118.
9. Cotelli M, Manenti R, Cappa SF, et al. Effect of transcranial magnetic stimulation on action naming in patients with Alzheimer disease. *Arch Neurol*. 2006;63: 1602–1604.
10. Cotelli M, Manenti R, Cappa SF, Zanetti O, Miniussi C. Transcranial magnetic stimulation improves naming in Alzheimer disease patients at different stages of cognitive decline. *Eur J Neurol*. 2008;15:1286–1292.
11. Cotelli M, Calabria M, Manenti R, et al. Improved language performance in Alzheimer disease following brain stimulation. *J Neurol Neurosurg Psychiatry*. 2010;82:794–797.
12. Ahmed MA, Darwish ES, Khedr EM, El Serogy YM, Ali AM. Effects of low versus high frequencies of repetitive transcranial magnetic stimulation on cognitive function and cortical excitability in Alzheimer's dementia. *J Neurol*. 2011; 259:83–92.
13. Bentwich J, Dobronevsky E, Aichenbaum S, et al. Beneficial effect of repetitive transcranial magnetic stimulation combined with cognitive training for the treatment of Alzheimer's disease: a proof of concept study. *J Neural Transm*. 2011; 118:463–471.
14. Rabey JM, Dobronevsky E, Aichenbaum S, Gonen O, Marton RG, Khaigrekht M. Repetitive transcranial magnetic stimulation combined with cognitive training is a safe and effective modality for the treatment of Alzheimer's disease: a randomized, double-blind study. *J Neural Transm*. 2013;120(5):813–819.
15. Devi G, Voss HU, Levine D, et al. Open-label, short-term, repetitive transcranial magnetic stimulation in patients with Alzheimer's disease with functional imaging correlates and literature review. *Am J Alzheimers Dis Other Demen*. 2014; 29(3):248–255.
16. Boggio PS, Valasek CA, Campanha C, et al. Non-invasive brain stimulation to assess and modulate neuroplasticity in Alzheimer's disease. *Neuropsychol Rehabil*. 2011;21(5):703–716.
17. Nardone R, Bergmann J, Christova M, et al. Effect of transcranial brain stimulation for the treatment of Alzheimer disease: a review. *Int J Alzheimers Dis*. 2012; 2012:5.
18. Rutherford G, Gole R, Moussavi Z. rTMS as a treatment of Alzheimer's disease with and without comorbidity of depression: a review. *Neurosci J*. 2013;2013:5.
19. Nardone R, Tezzon F, Holler Y, Golaszewski S, Trinka E, Brigo F. Transcranial magnetic stimulation (TMS)/repetitive TMS in mild cognitive impairment and Alzheimer's disease. *Acta Neurol Scand*. 2014;129(6):351–366.
20. Goldberg E. *The Executive Brain: Frontal Lobes and the Civilized Mind*. Oxford: Oxford University Press; 2002.
21. Robinson SR. Neuronal expression of glutamine synthetize in Alzheimer's disease indicates a profound impairment of metabolic interactions with astrocytes. *Neuochem Int*. 2000;36(4–5):471–482.
22. Michael N, Gösling M, Reutemann M, et al. Metabolic changes after repetitive transcranial magnetic stimulation (rTMS) of the left prefrontal cortex: a sham-controlled proton magnetic resonance spectroscopy (1H MRS) study of healthy brain. *Eur J Neurosci*. 2003;2(17):2462–2468.
23. Kimbrell TA, Dunn RT, George MS, et al. Left prefrontal rTMS and regional cerebral glucose metabolism in normal volunteers. *Psychiatry Res*. 2002;115(3): 101–113.
24. Brighina F, Palermo A, Daniele O, Alosisio A, Fierro B. High-frequency transcranial magnetic stimulation on motor cortex of patients affected by migraine with aura: a way to restore normal cortical excitability? *Cephalagia*. 2010;30(1): 46–52.
25. Cohen H, Kaplan Z, Kotler M, Kouperman I, Moisa R, Grisaru N. Repetitive transcranial magnetic stimulation of the right dorsolateral prefrontal cortex in posttraumatic stress disorder: a double-blind, placebo-controlled study. *Am J Psychiatry*. 2004;161(3):515–524.
26. Bidzan M, Bidzan L. Neurobehaviroal manifestation in early period of Alzheimer disease and vascular dementia. *Psychatr Pol*. 2014;48(2):319–330.
27. Wergeland JN, Selbæk G, Høgset LD, Söderhamn U, Kirkevold Ø. Dementia, neuropsychiatric symptoms and the use of psychotripic drugs among older people who receive domiciliary care: a cross-sectional study. *Int Psychogeriatr*. 2014; 26(3):383–291.
28. DaSilva AF, Volz MS, Bikson M, Fregni F. Electrode positioning and montage in transcranial direct current stimulation. *J Vis Exp*. 2011;51:2744.
29. Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. *Am J Psychiatry*. 1984;141(11):1356–1364.
30. Teri L, Truax P, Logsdon R, Uomoto J, Zarit S, Vitaliano PP. Assessment of behavioral problems in dementia: the revised memory and behavior problems checklist. *Psychol Aging*. 1992;7(4):622.
31. Nasreddine ZS, Phillips NA, Bédirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. 2005;53(4):695–699.
32. Garcia-Campuzano MT, Virues-Ortega J, Smith S, Moussavi Z. Effect of cognitive training targeting associative memory in the elderly: a small randomized trial and a longitudinal evaluation. *J Am Geriatr Soc*. 2013;61(12):2252–2254.
33. Kim HY. Statistical notes for clinical researchers: assessing normal distribution (2) using skewness and kurtosis. *Restor Dent Endod*. 2013;38(1):52–54.
34. Holland D, Desikan RS, Dale AM, McEvoy LK. Alzheimer's disease neuroimaging initiative. Rates of decline in Alzheimer disease decrease with age. *PloS One*. 2012;7(8):e42325.
35. Roalf DR, Moberg PJ, Xie SX, Wolk DA, Moelter ST, Arnold SE. Comparative accuracies of two common screening instruments for classification of Alzheimer's disease, mild cognitive impairment, and healthy aging. *Alzheimer Dement*. 2013; 9(5):529–537.
36. Lomarev MP, Kanchana S, Bara-Jimenez W, Iyer M, Wassermann EM, Hallett M. Placebo-controlled study of rTMS for the treatment of Parkinson's disease. *Mov Disord*. 2006;21(3):325–331.
37. Ueyama E, Ukai S, Ogawa A, et al. Chronic repetitive transcranial magnetic stimulation increases hippocampal neurogenesis in rats. *Psychiatry Clin Neurosci*. 2011;65(1):77–81.
38. Yoon KJ, Lee YT, Han TR. Mechanism of functional recovery after repetitive transcranial magnetic stimulation (rTMS) in the subacute cerebral ischemic rat model: neural plasticity or anti-apoptosis? *Exp Brain Res*. 2011;214(4):549–556.