

Study Result Summary

Study Title: Effect of Repetitive Transcranial Magnetic Stimulation (rTMS) on Serum Neurofilament Light Chain (sNfL) and its correlation to Cognitive function in Multiple Sclerosis

1. Participant Flow

- The study assessed 57 patients for suitability; 45 met the inclusion criteria and were randomized equally into three groups (15 per group).
- All randomized participants received the allocated intervention and were included in the primary analysis, with zero dropouts.
- All 45 patients completed the study and follow-up.

2. Recruitment Period

- Recruitment was conducted at the Outpatient Clinics of Neurology at Al-Azhar University Hospital and Assiut Health Insurance Hospital.
- Recruitment began in May 2022 and was completed in April 2024.
- Follow-up was completed by August 2024, with all participants followed for three months after the intervention.

3. Baseline Characteristics

- Demographic and clinical traits were evenly distributed among the three groups.
- The majority of participants were female, with mean ages ranging from 34.5 to 36.4 years.
- No statistically significant differences were observed across groups regarding age, sex, education, residence, marital status, illness duration, or baseline EDSS scores ($P > 0.05$).
- Disease-modifying therapy distribution differed significantly ($P < 0.001$).

4. Outcome Measures & Effects

- **Sustained Attention (SDMT):** Active rTMS groups showed significant short-term improvement, with Group A (Lt. DLPFC) showing the greatest increase, but effects were not maintained at the 3-month follow-up.
- **Verbal Learning and Memory (CVLT-II):** Significant short-term improvement in active groups, which also returned to baseline levels at the 3-month follow-up.
- **Visuospatial Memory and Learning (BVM-T-R):** Active rTMS groups showed transient improvement; Group A exhibited higher scores than the sham group post-treatment, but benefits were not maintained.
- **Serum Neurofilament Light Chain (sNfL):** No significant main effect of time, group, or interaction was found, indicating sNfL levels remained stable and were not influenced by rTMS.

5. Harms/Adverse Events

- No deaths or serious adverse events occurred.
- Three participants reported mild, transient tension-type headaches (two in Group A, one in Group B), which resolved without treatment interruption.

Table 1. Baseline demographic and clinical characteristics of the studied groups (N = 45)

Parameter	Group A (n = 15)	Group B (n = 15)	Group C (n = 15)	P value
Age (years)	35.0 ± 11.9	36.4 ± 8.5	34.5 ± 8.9	0.86
Educational years	9.7 ± 7.2	11.7 ± 5.1	11.2 ± 6.1	0.64
Gender				0.43
Male	5 (33.3%)	4 (26.7%)	2 (13.3%)	
Female	10 (66.7%)	11 (73.3%)	13 (86.7%)	
Residence				0.07
Urban	10 (66.7%)	6 (40.0%)	12 (62.2%)	
Rural	5 (33.3%)	9 (60.0%)	3 (37.8%)	
Educational level				0.41
Illiterate	5 (33.3%)	2 (13.3%)	3 (20.0%)	
Secondary	3 (20.0%)	8 (53.3%)	6 (40.0%)	
Post-secondary	7 (46.7%)	5 (33.3%)	6 (40.0%)	
Marital status				0.24
Married	9 (60.0%)	13 (86.7%)	13 (86.7%)	
Single	5 (33.3%)	2 (13.3%)	1 (6.7%)	
Widowed	1 (6.7%)	0 (0.0%)	1 (6.7%)	
Disease duration (years)	6.5 ± 2.7	5.9 ± 2.9	6.5 ± 3.1	0.78
EDSS score	2.8 ± 1.2	3.2 ± 1.3	3.0 ± 1.0	0.68
Disease-modifying therapy (DMT)				<0.001
Interferon β-1a	11 (73.3%)	10 (66.7%)	1 (6.7%)	
Fingolimod	3 (20.0%)	4 (26.7%)	1 (6.7%)	
Ocrelizumab	1 (6.7%)	0 (0.0%)	2 (13.3%)	

Rituximab	0 (0.0%)	1 (6.7%)	0 (0.0%)
Natalizumab	0 (0.0%)	0 (0.0%)	1 (6.7%)
Dimethyl fumarate	0 (0.0%)	0 (0.0%)	2 (13.3%)
Not started yet	0 (0.0%)	0 (0.0%)	8 (53.3%)

Data presented as mean $\pm$ standard deviation (SD) or frequency (percentage).

Comparisons between groups were performed using one-way analysis of variance (ANOVA) for continuous variables and Chi-square test or Fisher's exact test, as appropriate, for categorical variables. A P value < 0.05 was considered statistically significant. EDSS, Expanded Disability Status Scale; DMT, disease-modifying therapy; SD, standard deviation

Table 2: Symbol Digit Modalities Test (SDMT) findings at baseline, after 10 sessions and after three months of sessions. Total N=45

Parameter	Group A (Lt. DLPFC)(n=15)	Group B (Vertex)(n=15)	Group C (Sham)(n=15)
Baseline	27.57 $\pm$ 6.30	28.87 $\pm$ 8.72	29.70 $\pm$ 12.51
After 10 sessions	48.68 $\pm$ 7.73*,†	38.86 $\pm$ 10.98*	27.85 $\pm$ 10.27
After 3 months	29.40 $\pm$ 3.52	31.63 $\pm$ 10.90	28.28 $\pm$ 10.05
Statistical Analysis (RM-ANOVA)	F-value	P-value	Partial Eta Squared (η^2)
Time effect (df=2,84)	16.827	<0.001	0.286 (Large Effect)
Group effect (df=2,42)	4.649	0.015	0.181 (Large Effect)
Time $\times$ Group interaction (df=4,84)	7.69	<0.001	0.268 (Large Effect)

Data are presented as mean $\pm$ standard deviation (SD). Repeated-measures ANOVA was used to assess the effects of time, group, and group-by-time interaction. Partial Eta Squared (η^2) is reported as a measure of effect size. * Indicates a statistically significant improvement compared to baseline within the same group (P < 0.05 with Bonferroni adjustment). † Indicates a statistically significant difference compared to Group C (Sham) at that specific time point (P = 0.014 with Bonferroni adjustment).

Table 3. The Symbol Digit Modalities Test (SDMT) Pairwise comparisons at baseline, after 10 sessions and after three months of sessions in groups A, B and C.(N=45)

Pairwise Comparisons

Measure: SDMT							
Group	(I) followup	(J) followup	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
A	1	2	-21.116*	3.427	<.001	-29.663	-12.570
		3	-1.833	3.410	1.000	-10.337	6.672
	2	1	21.116*	3.427	<.001	12.570	29.663
		3	19.284*	2.692	<.001	12.570	25.997
	3	1	1.833	3.410	1.000	-6.672	10.337
		2	-19.284*	2.692	<.001	-25.997	-12.570
B	1	2	-9.988*	3.427	.017	-18.534	-1.442
		3	-2.763	3.410	1.000	-11.267	5.742
	2	1	9.988*	3.427	.017	1.442	18.534
		3	7.225*	2.692	.031	.512	13.939
	3	1	2.763	3.410	1.000	-5.742	11.267
		2	-7.225*	2.692	.031	-13.939	-.512
C	1	2	1.851	3.427	1.000	-6.696	10.397
		3	1.422	3.410	1.000	-7.082	9.927
	2	1	-1.851	3.427	1.000	-10.397	6.696
		3	-.428	2.692	1.000	-7.142	6.285
	3	1	-1.422	3.410	1.000	-9.927	7.082
		2	.428	2.692	1.000	-6.285	7.142

Followup 1: at baseline, Followup 2: after 10 sessions, Followup 3: after 3 months of sessions,

I: subtracted from it: subtracted.

Table 4: California verbal learning test (CVLT-II) findings at baseline, after 10 sessions and after three months of sessions. Total N=45

Parameter	Group A (Lt. DLPFC)(n=15)	Group B (Vertex)(n=15)	Group C (Sham)(n=15)
Baseline	40.07±8.51	33.67±9.30	38.73±6.80
After 10 sessions	57.33±7.93*,†	40.33±12.72*	37.71±5.40
After 3 months	41.93±7.78	35.06±11.20	37.65±4.53

Statistical Analysis (RM-ANOVA)	F-value	P-value	Partial Eta Squared (η^2)
Time effect (df=2,84)	23.986	<0.001	0.363 (Large Effect)
Group effect (df=2,42)	8.478	0.001	0.288 (Large Effect)
Time $\times$ Group interaction (df=4,84)	11.085	<0.001	0.345 (Large Effect)

Data are presented as mean $\pm$ standard deviation (SD). Repeated-measures ANOVA was used to assess the effects of time, group, and group-by-time interaction. Partial Eta Squared (η^2) is reported as a measure of effect size. * Indicates a statistically significant improvement compared to baseline within the same group. † Indicates a statistically significant difference compared to Group C (Sham) at that specific time point.

Table 5. California verbal learning test (CVLT-II) Pairwise comparisons at baseline, after 10 sessions and after three months of sessions in groups A, B and C.(N=45)

Pairwise Comparisons							
Measure: CVLT							
Group	(I) followup	(J) followup	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
A	1	2	-17.267*	2.419	<.001	-23.299	-11.234
		3	-1.867	2.150	1.000	-7.229	3.495
	2	1	17.267*	2.419	<.001	11.234	23.299
		3	15.400*	1.689	<.001	11.188	19.612
	3	1	1.867	2.150	1.000	-3.495	7.229
		2	-15.400*	1.689	<.001	-19.612	-11.188
B	1	2	-6.667*	2.419	.026	-12.699	-.634
		3	-1.396	2.150	1.000	-6.758	3.966
	2	1	6.667*	2.419	.026	.634	12.699
		3	5.271*	1.689	.010	1.059	9.482
	3	1	1.396	2.150	1.000	-3.966	6.758
		2	-5.271*	1.689	.010	-9.482	-1.059
C	1	2	1.026	2.419	1.000	-5.007	7.058
		3	1.081	2.150	1.000	-4.281	6.443
	2	1	-1.026	2.419	1.000	-7.058	5.007
		3	.055	1.689	1.000	-4.156	4.267

	3	1	-1.081	2.150	1.000	-6.443	4.281
		2	-.055	1.689	1.000	-4.267	4.156

Followup 1: at baseline, Followup 2: after 10 sessions, Followup 3: after 3 months of sessions. I: subtracted from it: subtracted.

Table 6: Brief Visuospatial Memory Test–Revised BVMT findings at baseline, after 10 sessions and after three months of sessions. Total N=45

Parameter	Group A (Lt. DLPFC)(n=15)	Group B (Vertex)(n=15)	Group C (Sham)(n=15)
Baseline	20.24±3.19	19.04±4.96	19.05±6.37
After 10 sessions	27.44±4.40*,†	23.27±5.54*	17.17±5.66
After 3 months	20.59±3.03	17.82±4.85	14.26±4.29
Statistical Analysis (RM-ANOVA)	F-value	P-value	Partial Eta Squared (η^2)
Time effect (df=2,84)	26.598	<0.001	0.388 (Large Effect)
Group effect (df=2,42)	8.379	0.001	0.285 (Large Effect)
Time × Group interaction (df=4,84)	7.294	<0.001	0.258 (Large Effect)

Data are presented as mean ± standard deviation (SD). Repeated-measures ANOVA was used to assess the effects of time, group, and group-by-time interaction. Partial Eta Squared (η^2) is reported as a measure of effect size. * Indicates a statistically significant improvement compared to baseline within the same group. † Indicates a statistically significant difference compared to Group C (Sham) at that specific time point.

Table 7. Brief Visuospatial Memory Test–Revised test (BVMT-R) Pairwise comparisons at baseline, after 10 sessions and after three months of sessions in groups A, B and C.(N=45)

Pairwise Comparisons							
Measure: BVMT							
Group	(I) followup	(J) followup	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
A	1	2	-7.206*	1.353	<.001	-10.579	-3.833
		3	-.351	1.116	1.000	-3.134	2.431

	2	1	7.206*	1.353	<.001	3.833	10.579
		3	6.855*	1.170	<.001	3.938	9.771
	3	1	.351	1.116	1.000	-2.431	3.134
		2	-6.855*	1.170	<.001	-9.771	-3.938
	B	1	-4.224*	1.353	.010	-7.597	-.851
			1.220	1.116	.842	-1.563	4.002
		2	4.224*	1.353	.010	.851	7.597
			5.444*	1.170	<.001	2.527	8.360
		3	-1.220	1.116	.842	-4.002	1.563
			-5.444*	1.170	<.001	-8.360	-2.527
C	1	2	1.882	1.353	.514	-1.491	5.255
		3	4.792*	1.116	<.001	2.009	7.574
	2	1	-1.882	1.353	.514	-5.255	1.491
		3	2.910	1.170	.051	-.007	5.826
	3	1	-4.792*	1.116	<.001	-7.574	-2.009
		2	-2.910	1.170	.051	-5.826	.007

Followup 1: at baseline, Followup 2: after 10 sessions, Followup 3: after 3 months of sessions,

I: subtracted from it: subtracted.

Table 8. sNeurofilament light chain (sNfL) findings at baseline, after 10 sessions and after three months of sessions. Total N=45

Parameter	Group A (Lt. DLPFC)(n=15)	Group B (Vertex)(n=15)	Group C (Sham)(n=15)
Baseline	3.89±0.58	4.05±3.84	3.83±2.82
After 10 sessions	3.63±0.32	3.70±2.23	3.65±2.13
After 3 months	3.69±1.08	3.94±2.69	3.46±2.25
Statistical Analysis (RM-ANOVA)	F-value	P-value	Partial Eta Squared (η^2)
Time effect (df=1.12,46.90)	0.278	0.814	0.006 (Small Effect)
Group effect (df=2,42)	0.082	0.922	0.004 (Small Effect)
Time × Group interaction (df=2.23,46.90)	0.056	0.753	0.002 (Small Effect)

Data are presented as mean $\pm$ standard deviation (SD). Repeated-measures ANOVA was used to assess the effects of time, group, and group-by-time interaction. Partial Eta Squared (η^2) is reported as a measure of effect size. No statistically significant differences were observed across time points or between study groups.

Figure 1: CONSORT flow diagram depicting the progress of participants through each stage of the randomized controlled trial, including enrollment, allocation, follow-up, and analysis.

