RESEARCH PAPER

Comparative Effects of 10-Hz rTMS with and Without Alpha Binaural Beats on Post-Stroke Cognitive Function

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ABSTRACT

Background: Post-stroke cognitive impairment is common, and effective adjunctive neuromodulation strategies are needed. This study compared 10-Hz rTMS alone with alpha binaural beat combined with 10-Hz rTMS in patients with subacute ischemic stroke.

Methods: In this randomized pretest–posttest study, 45 patients were allocated equally to standard therapy, 10-Hz rTMS, or combined alpha binaural beat and 10-Hz rTMS. Cognitive function and qEEG-derived DTABR were assessed at baseline, after 10 sessions, and after 20 sessions.

Results: Cognitive improvement differed significantly among groups after 10 and 20 sessions (both $p<0.001$). Both active interventions produced greater early cognitive improvement than standard therapy, but the combination was not superior to rTMS alone. After 10 sessions, DTABR decreased in the combined group and increased in the control group, with a significant between-group difference ($p=0.011$); this effect was not sustained at 20 sessions. Changes in MoCA-Ina and DTABR were inversely correlated after 10 sessions ($\rho=-0.430$, adjusted $p=0.006$).

Conclusion: 10-Hz rTMS was associated with early cognitive improvement, while adding alpha binaural beats provided no additional clinical benefit. The combination produced transient neurophysiological modulation, suggesting a potential early effect on cortical oscillatory activity without sustained additive efficacy.

Keywords: post-stroke cognitive impairment; 10-Hz rTMS; alpha binaural beats; cognitive function; qEEG; DTABR.

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INTRODUCTION

Stroke remains a major cause of death and long-term disability worldwide, with ischemic stroke accounting for the majority of stroke events and imposing a substantial burden on healthcare systems [1]. Post-stroke cognitive impairment (PSCI) is a frequent and clinically important consequence of stroke, affecting multiple domains including attention, memory, executive function, language, and visuospatial processing, with substantial implications for independence, quality of life, and rehabilitation outcomes [2]. Although spontaneous recovery and conventional rehabilitation may improve cognitive function, recovery can be incomplete or plateau over time, highlighting the need for adjunctive neurorestorative strategies [3]. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulation technique capable of modifying cortical excitability and functional networks involved in cognition [4]. High-frequency rTMS, particularly at 10 Hz and targeting the dorsolateral prefrontal cortex, has demonstrated beneficial effects on global cognitive function in patients with PSCI [2].

Binaural beat stimulation represents another non-invasive approach that may influence cortical oscillatory activity through frequency-specific auditory stimulation. Alpha-frequency binaural beats, particularly around 10 Hz, have been associated with increased alpha activity, reduced theta activity, and improvements in selected cognitive performance measures [5]. However, evidence for binaural beat stimulation in stroke populations remains limited, and recent work suggests that its neurophysiological effects may vary according to stimulation frequency and clinical context [6]. Importantly, whether alpha binaural beats can enhance the therapeutic effects of 10-Hz rTMS in patients with post-stroke cognitive impairment remains unclear. This knowledge gap is particularly relevant because

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multimodal neuromodulation may provide complementary effects on cortical excitability and oscillatory activity, potentially augmenting cognitive recovery beyond rTMS alone [7].

Clinical and neurophysiological measures may provide complementary information regarding treatment response in PSCI. The Indonesian version of the Montreal Cognitive Assessment (MoCA-Ina) enables multidomain assessment of cognitive function, whereas quantitative electroencephalography (qEEG) provides an objective measure of treatment-related changes in brain electrical activity [8]. The delta-theta/alpha-beta ratio (DTABR) may further characterize the balance between slower and faster EEG activity and has been proposed as a neurophysiological marker of cognitive dysfunction and recovery [9]. Serial assessment is therefore valuable for distinguishing early treatment responses from cumulative effects after repeated stimulation. Accordingly, this study aimed to compare the effects of 10-Hz rTMS alone versus alpha binaural beat combined with 10-Hz rTMS on cognitive function in patients with subacute ischemic stroke, assessed through serial changes in MoCA-Ina scores and qEEG-derived DTABR after 10 and 20 treatment sessions.

MATERIALS AND METHODS

Study Design and Setting

This quantitative true experimental study employed a randomized pretest–posttest controlled-group design. The study was conducted at Hasanuddin University Hospital, Makassar, Indonesia, and its affiliated hospitals from March 2026 until the required sample size was achieved. The target population comprised patients with subacute ischemic stroke and cognitive impairment, while the accessible population consisted of eligible outpatients receiving follow-up care at the study hospitals.

Participants and Eligibility

Participants were eligible if they had a first-ever ischemic stroke with an onset of 7 days to 3 months, were aged 30–65 years, had completed at least 9 years of formal education, and had cognitive impairment indicated by a MoCA-Ina score <26. Stroke diagnosis was established based on clinical assessment and neuroimaging findings on computed tomography or magnetic resonance imaging. Participants were also required to provide written informed consent and to complete the cognitive assessment, qEEG recording, and assigned intervention procedures.

Patients were excluded if they had aphasia, pre-existing dementia, seizures or a history of epilepsy, previous traumatic brain injury, pregnancy, hearing impairment, a cardiac pacemaker, cochlear implant, or intracranial metallic implants such as surgical clips. Participants were considered to have withdrawn from the study if they voluntarily discontinued participation, attended fewer than 80% of scheduled intervention sessions, or died during the study period.

Sample Size and Sampling

The minimum sample size was calculated for comparison of two independent numerical means using a two-sided significance level of 5%, 80% statistical power, a standard deviation of 0.69, and a minimum clinically meaningful mean difference of 0.72. The calculation yielded a minimum of 15 participants per group. To preserve the required sample size and statistical power, replacement recruitment was performed when a participant withdrew or met the dropout criteria. Eligible participants were recruited consecutively and subsequently randomized into three study groups.

Randomization and Study Groups

After baseline assessment, participants were randomly allocated to one of three groups: standard therapy with sham stimulation, 10-Hz rTMS with sham auditory stimulation, or combined alpha binaural beat and 10-Hz rTMS. All participants continued to receive standard medical management for subacute ischemic stroke according to the applicable hospital clinical practice guideline, including antiplatelet therapy, statins, antihypertensive treatment when indicated, and citicoline. Medication adherence was monitored using medical records and pill counts.

The control procedure was designed to mimic the duration and general experience of active treatment without delivering active magnetic or auditory stimulation. Sham auditory stimulation consisted of neutral or silent audio, while sham magnetic stimulation was performed using the corresponding sham procedure.

Intervention Protocol

The intervention consisted of 20 sessions delivered five times per week over 4 weeks. Active stimulation was applied to the left dorsolateral prefrontal cortex using 10-Hz rTMS. Stimulation intensity was set according to the predefined protocol based on the resting motor threshold. The rTMS-only group received active magnetic stimulation accompanied by sham audio, whereas the combination group received 10-Hz alpha binaural beat stimulation through stereo headphones followed by active rTMS. The alpha binaural beat was delivered at 10 Hz for 30 min before rTMS. The control group underwent the corresponding sham procedures while continuing standard therapy.

Cognitive Assessment

Cognitive function was assessed using the Indonesian version of the Montreal Cognitive Assessment (MoCA-Ina), which evaluates multiple cognitive domains and was also used to identify cognitive impairment at screening. Assessments were performed at baseline, after 10 intervention sessions, and after 20 sessions. The primary clinical outcome was the change in MoCA-Ina score from baseline to sessions 10 and 20.

Quantitative Electroencephalography

qEEG recordings were obtained at baseline, after 10 sessions, and after 20 sessions. Before recording, participants were instructed to wash their hair and scalp

with shampoo and avoid using conditioner, hair oil, or styling products. Recordings were performed in a quiet, dimly lit room with participants in a relaxed supine position and instructed to remain awake.

EEG activity was recorded using a Cadwell Easy III system with 19 electrodes positioned according to the International 10–20 System. Data acquisition was initiated after electrode impedance was below 20 Ω, using a low-cut filter of 0.53 Hz, a high-cut filter of 70 Hz, and an activated notch filter. Each recording lasted 10 min with the participant's eyes closed while remaining awake. Raw EEG data were processed using NeuroGuide Deluxe qEEG software. Artifact detection and removal were performed before analysis, and a minimum of 10 s of artifact-free EEG data was selected for analysis. The principal neurophysiological outcome was the delta-plus-theta to alpha-plus-beta ratio (DTABR), calculated as an index of the relative predominance of slow- versus fast-frequency activity.

Outcome Measures

The primary clinical outcome was the change in MoCA-Ina score, calculated as the post-intervention score minus the baseline score (ΔMoCA). The primary neurophysiological outcome was the corresponding change in DTABR (ΔDTABR). Outcomes were evaluated at both 10 and 20 sessions to characterize early and cumulative treatment responses. The relationship between clinical and neurophysiological responses was assessed by examining the correlation between ΔMoCA and ΔDTABR.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 29.0. Categorical variables were summarized as frequencies and percentages, whereas continuous variables were expressed as mean ± standard deviation for normally distributed data or median (minimum–maximum) for non-normally distributed data. Normality was assessed using the Shapiro–Wilk test. Baseline comparability among the three groups was assessed using the Kruskal–Wallis test for non-normally distributed continuous variables and Fisher's exact test for categorical variables.

Within-group changes in MoCA-Ina across baseline, session 10, and session 20 were assessed using the Friedman test, followed by Wilcoxon signed-rank tests with Bonferroni correction when the overall comparison was significant. Changes in DTABR between baseline and sessions 10 and 20 were assessed using the Wilcoxon signed-rank test. Between-group differences in ΔMoCA and ΔDTABR were evaluated using the Kruskal–Wallis test, followed by pairwise Mann–Whitney U tests with Bonferroni correction when appropriate. Associations between ΔMoCA and ΔDTABR were assessed using Spearman's rank correlation for baseline-to-session-10 and baseline-to-session-20 changes. All tests were two-sided, and $p<0.05$ was considered statistically significant.

Ethical Considerations

The study was approved by the Biomedical Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (No. 747/UN4.6.4.5.31/PP36/2026). All participants received an explanation of the study objectives, procedures, potential benefits, and risks before enrollment. Written informed consent was obtained from all participants before any study-related procedures were performed. Standard medical care was maintained throughout the study.

RESULTS

Baseline Characteristics

A total of 45 patients with subacute ischemic stroke and cognitive impairment were included and equally allocated to the control, rTMS, and ABB+rTMS groups, with 15 participants in each group. The groups were comparable in terms of age, sex, educational attainment, and vascular risk factors, with no statistically significant between-group differences (all $p>0.05$). Baseline cognitive performance was also comparable, with median MoCA-Ina scores of 19, 21, and 15 in the control, rTMS, and ABB+rTMS groups, respectively ($p=0.163$) (Table 1).

Changes in Cognitive Function

MoCA-Ina scores increased over time in all three groups. At session 10, median scores were 20, 23, and 22 in the control, rTMS, and ABB+rTMS groups, respectively, increasing to 21, 24, and 24 at session 20. However, absolute scores did not differ significantly between groups at session 10 ($p=0.221$) or session 20 ($p=0.225$) (Table 2). Within-group analysis showed significant longitudinal changes in all three groups by the Friedman test. After Bonferroni correction, the rTMS group showed significant improvement from baseline to sessions 10 and 20, whereas the ABB+rTMS group showed significant improvement from baseline to both time points and an additional significant improvement between sessions 10 and 20. No pairwise comparison remained significant in the control group (Table 3).

Between-Group Differences in Cognitive Improvement

The magnitude of cognitive improvement differed significantly among the three groups after both 10 and 20 sessions. The median ΔMoCA-Ina was 0, 5, and 5 points after 10 sessions and 0, 6, and 6 points after 20 sessions in the control, rTMS, and ABB+rTMS groups, respectively (both $p<0.001$) (Table 4). After 10 sessions, both rTMS and ABB+rTMS produced greater cognitive improvement than the control group (adjusted $p=0.002$ and <0.001, respectively), whereas the difference between the two active interventions was not significant (adjusted $p=0.670$). After 20 sessions, ABB+rTMS remained significantly different from the control group (adjusted $p<0.001$), while neither the control versus rTMS comparison (adjusted $p=0.073$) nor the rTMS versus ABB+rTMS comparison (adjusted $p=0.242$) was significant.

Changes in Neurophysiological Activity

DTABR values did not differ significantly between groups at baseline, after 10 sessions, or after 20 sessions (p=0.536, p=0.431, and p=0.402, respectively) (Table 5). Within-group analysis showed a significant increase in DTABR after 10 sessions in the control group (p=0.008), whereas the ABB+rTMS group demonstrated a significant reduction (p=0.009). No significant change was observed in the rTMS group. After 20 sessions, none of the groups showed a significant change from baseline (Table 6).

Between-Group Differences in DTABR

After 10 sessions, the median ΔDTABR was +0.684 in the control group, −1.106 in the rTMS group, and −3.239 in the ABB+rTMS group, with a significant overall between-group difference (H=8.933, p=0.011). Post hoc analysis showed a significant difference only between the control and ABB+rTMS groups (adjusted p=0.003), whereas the control versus rTMS (adjusted p=0.513) and rTMS versus ABB+rTMS (adjusted p=1.000) comparisons were not significant. After 20 sessions, the between-group difference was no longer significant (H=4.161, p=0.125) (Table 7).

Association Between Cognitive and Neurophysiological Changes

A moderate inverse correlation was observed between ΔMoCA-Ina and ΔDTABR during the first 10 sessions (Spearman's ρ=−0.430, p=0.003; Bonferroni-adjusted p=0.006). At 20 sessions, the correlation remained inverse but was weaker and did not remain statistically significant after adjustment (ρ=−0.328, p=0.028; adjusted p=0.056) (Table 8).

DISCUSSION

This study found that both active interventions were associated with greater early cognitive improvement than standard therapy, whereas adding alpha binaural beats did not provide a significant advantage over 10-Hz rTMS alone. The cognitive benefit of rTMS is consistent with Christiantoro et al. (2019) [10], who reported improved cognitive performance following left dorsolateral prefrontal cortex stimulation, and with Yin et al. (2020), who demonstrated cognitive and functional benefits after 10-Hz stimulation of the same target [11]. Similar findings were reported by Kim et al. (2022) in patients with subacute stroke [12]. These effects may reflect enhanced cortical excitability and recruitment of residual frontoparietal and frontostriatal networks involved in cognitive processing [13].

The response pattern suggests that the principal cognitive benefit of rTMS emerged during the early treatment phase, with limited additional improvement thereafter. This is consistent with Tsai et al. (2020) [14], supporting the concept that repeated neuromodulation may produce an early gain followed by a relative plateau. Importantly, longitudinal improvement within an active group does not establish superiority over standard care, particularly during the subacute phase when spontaneous recovery and rehabilitation may contribute to cognitive change [15]. The present between-group findings therefore provide stronger support for the early efficacy of active stimulation than within-group changes alone.

The combined intervention produced an early reduction in DTABR, suggesting modulation of cortical oscillatory activity, but this effect was not sustained and did not translate into superior cognitive improvement compared with rTMS alone. This observation is partly consistent with Mujib et al. (2021) [5], who reported that 10-Hz alpha binaural stimulation altered alpha and theta activity and frontoparietal connectivity. However, Zhong et al. (2025) found inconsistent neurophysiological effects of binaural stimulation in stroke, indicating that auditory entrainment may depend on stimulation frequency and individual brain state [16]. Thus, the present findings do not support a clear additive effect of alpha binaural beats when combined with 10-Hz rTMS.

The lack of a significant DTABR change after rTMS despite cognitive improvement indicates that cognitive recovery cannot be represented by a single global spectral measure. rTMS-related neuroplasticity may instead involve functional connectivity, network efficiency, or compensatory recruitment, as demonstrated by Yin et al. (2020) [11]. Conversely, the transient DTABR response following combined treatment suggests that qEEG may capture early physiological changes that do not necessarily persist as clinical recovery progresses. This distinction supports the complementary use of cognitive and neurophysiological outcomes.

Overall, the findings indicate that 10-Hz rTMS provides clinically meaningful early cognitive benefits in subacute post-stroke cognitive impairment, while the addition of alpha binaural beats may influence early cortical oscillatory activity without demonstrable additional clinical efficacy. The inverse association between early changes in MoCA-Ina and DTABR further suggests that reduced slow-wave predominance may accompany cognitive recovery, although this relationship was not sustained at the later assessment. These results support 10-Hz rTMS as a promising adjunctive intervention while indicating that the therapeutic role of alpha binaural beats requires further evaluation.

LIMITATIONS

This study has several limitations. Clinical heterogeneity, including lesion location, laterality, lesion burden, cognitive reserve, vascular comorbidities, and residual network integrity, was not fully stratified and may have contributed to variability in treatment response. The fixed 10-Hz alpha binaural beat protocol was not individualized according to individual alpha frequency, and direct target engagement was not verified. Differences in carrier frequency, auditory comfort, attention, and hearing status may also have influenced the response.

The neurophysiological assessment was susceptible to state-dependent factors such as drowsiness, arousal, medication effects, eye condition, and recording artifacts, while regional changes in DTABR were not separately evaluated. MoCA-Ina provides a global cognitive measure

and may be affected by practice effects without identifying specific cognitive domains or neural networks responsible for recovery. In addition, follow-up ended after 20 sessions, preventing assessment of the durability of treatment effects.

The correlation between cognitive and neurophysiological changes was based on pooled groups and therefore should not be interpreted as evidence of individual-level causality. Finally, the findings may not be generalizable to older patients, acute or chronic-stage stroke, hemorrhagic stroke, or patients with more severe cognitive impairment. Future studies should incorporate lesion-based stratification, individualized alpha-frequency stimulation, direct verification of target engagement, domain-specific cognitive and functional outcomes, and longer follow-up to determine the durability and mechanisms of treatment response.

CONCLUSIONS

In patients with subacute post-stroke cognitive impairment, cognitive function improved over time across all groups, but active neuromodulation produced greater clinical improvement than standard therapy alone, particularly during the early treatment phase. Both 10-Hz rTMS and its combination with alpha binaural beats improved cognitive outcomes, with no significant evidence that adding alpha binaural beats provided an additional clinical benefit over rTMS alone. Neurophysiological changes were most evident during the early treatment period, with the combined intervention showing a greater reduction in DTABR than standard therapy, although this effect was not sustained or superior to rTMS alone at the later assessment. The early inverse association between cognitive improvement and DTABR further suggests that reduced slow-wave predominance may accompany cognitive recovery. Overall, 10-Hz rTMS appears to be a promising adjunctive approach for post-stroke cognitive rehabilitation, whereas alpha binaural beats may provide transient neurophysiological modulation without demonstrated additional clinical efficacy.

DECLARATIONS

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Conflict of Interest

The authors declare that they have no conflicts of interest relevant to this work.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to appropriate ethical and institutional considerations.

Consent for Publication

Not applicable. No individually identifiable participant information or images are included in this manuscript.

Author Contributions

MAO: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. SWG: Conceptualization, Methodology, Supervision, Validation, Writing – review and editing. JT: Methodology, Supervision, Validation, Writing – review and editing. MIB: Investigation, Data curation, Formal analysis, Writing – review and editing. CR: Investigation, Data curation, Writing – review and editing. All authors reviewed and approved the final manuscript.

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TABLES

Table 1. Baseline Characteristics of Participants According to Treatment Group

Characteristic	Control (n=15)	rTMS (n=15)	ABB+rTMS (n=15)	P-value
Age, years	58 (47–65)	53 (39–65)	62 (31–65)	0.900
Sex, n (%)				1.000
Male	11 (73.3)	11 (73.3)	10 (66.7)	
Female	4 (26.7)	4 (26.7)	5 (33.3)	
Education, n (%)				0.689
Junior high school	0 (0.0)	2 (13.4)	3 (20.0)	
Senior high school	5 (33.3)	5 (33.3)	5 (33.3)	
Diploma III	1 (6.7)	0 (0.0)	1 (6.7)	
Bachelor's degree	7 (46.7)	8 (53.3)	3 (20.0)	
Master's degree	0 (0.0)	0 (0.0)	2 (13.3)	
Doctoral degree	2 (13.3)	0 (0.0)	1 (6.7)	
Dyslipidemia, n (%)	5 (33.3)	3 (20.0)	0 (0.0)	0.071
Diabetes mellitus, n (%)	2 (13.3)	4 (26.7)	8 (53.3)	0.077
Hypertension, n (%)	12 (80.0)	13 (86.7)	9 (60.0)	0.311
Smoking, n (%)	1 (6.7)	1 (6.7)	3 (20.0)	0.594
Coronary heart disease, n (%)	3 (20.0)	0 (0.0)	2 (13.3)	0.343
Baseline MoCA-Ina score	19 (4–23)	21 (0–24)	15 (3–22)	0.163

Continuous variables are presented as median (minimum–maximum), and categorical variables as n (%). The Kruskal–Wallis test was used for age and baseline MoCA-Ina; categorical variables were analyzed using the exact test.

Table 2. MoCA-Ina Scores According to Treatment Group and Assessment Time

Assessment time	Group	Mean ± SD	Median (Min–Max)	P-value*

Baseline	Control	18.40 ± 4.88	19 (4–23)	0.163
	rTMS	15.80 ± 8.25	21 (0–24)	
	ABB+rTMS	13.40 ± 6.95	15 (3–22)	
Session 10	Control	18.73 ± 4.77	20 (4–22)	0.221
	rTMS	19.87 ± 8.68	23 (0–29)	
	ABB+rTMS	19.13 ± 5.03	22 (9–25)	
Session 20	Control	19.27 ± 4.96	21 (4–23)	0.225
	rTMS	20.80 ± 8.48	24 (0–30)	
	ABB+rTMS	20.93 ± 5.78	24 (9–28)	

Data are presented as mean ± standard deviation and median (minimum–maximum). *Kruskal–Wallis test.

Table 3. Changes in MoCA-Ina Scores Within Each Treatment Group

Group	Friedman p-value	Baseline–S10	Baseline–S20	S10–S20
Control	0.032*	1.000	0.144	0.115
rTMS	<0.001*	0.004*	0.006*	0.160
ABB+rTMS	<0.001*	0.002*	0.002*	0.028*

Friedman test was used for overall within-group comparisons, followed by Wilcoxon signed-rank tests with Bonferroni correction for pairwise comparisons. *p<0.05.

Table 4. Between-Group Comparison of Changes in MoCA-Ina Scores

Change	Control	rTMS	ABB+rTMS	H	P-value
ΔMoCA 0–10	0 (−2–4)	5 (0–11)	5 (2–10)	22.468	<0.001*
ΔMoCA 0–20	0 (−2–4)	6 (−2–12)	6 (4–13)	16.050	<0.001*

ΔMoCA represents the change in MoCA-Ina score from baseline. Kruskal–Wallis test; *p<0.05.

Table 5. DTABR Values According to Treatment Group and Assessment Time

Assessment time	Control	rTMS	ABB+rTMS	H	P-value
Baseline	8.884 (2.240–17.995)	6.895 (1.688–36.064)	11.573 (4.054–34.773)	1.246	0.536
After 10 sessions	9.805 (1.793–23.364)	8.881 (0.893–20.005)	7.109 (3.064–28.999)	1.684	0.431
After 20 sessions	8.982 (1.513–24.159)	5.978 (0.835–18.001)	8.574 (2.344–66.649)	1.825	0.402

Data are presented as median (minimum–maximum). Kruskal–Wallis test.

Table 6. Changes in DTABR Within Each Treatment Group

Group	Baseline	After 10 sessions	P (0–10)	After 20 sessions	P (0–20)
Control	8.884 (2.240–17.995)	9.805 (1.793–23.364)	0.008*	8.982 (1.513–24.159)	0.359
rTMS	6.895 (1.688–36.064)	8.881 (0.893–20.005)	0.524	5.978 (0.835–18.001)	0.252
ABB+rTMS	11.573 (4.054–34.773)	7.109 (3.064–28.999)	0.009*	8.574 (2.344–66.649)	0.679

Data are presented as median (minimum–maximum). Wilcoxon signed-rank test; *significant after Bonferroni correction (α=0.025).

Table 7. Correlation Between Changes in MoCA-Ina and DTABR

Period	Spearman's rs	P-value	Adjusted P-value	Direction/Strength
Δ0–10	−0.430	0.003	0.006*	Negative, moderate
Δ0–20	−0.328	0.028	0.056	Negative, weak

Spearman's rank correlation was used. P-values were adjusted using Bonferroni correction for two correlation analyses. *Adjusted p<0.05.