Brain Stimulation 19 (2026) 102976

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.journals.elsevier.com/brain-stimulation

Timing matters: Inverted U-shaped efficacy of dose distribution in translational neuromodulation for treatment-resistant depression

Dear Editor,

The advent of accelerated repetitive transcranial magnetic stimulation (a-rTMS)—defined as the administration of multiple daily sessions—has significantly advanced neuromodulation for treatment-resistant depression (TRD [1]). By shortening the treatment period, a-rTMS can reduce the time commitment and lead to quicker symptom improvement [2]. With multiple daily sessions, accelerated protocols vary in the temporal distribution of rTMS sessions, and certain configurations may prove more effective in restoring neurophysiological balance, potentially leading to improved outcomes in depressive disorders [3]. While the dose-response relationship has been relatively well-characterized [4], the influence of stimulation timing (i.e., sessions' temporal distribution) on clinical outcomes remains unclear. Protocol and patient heterogeneity in clinical a-rTMS may hinder identification of parameter–response relationships [2], underscoring the role of preclinical studies in helping to clarify determinants of efficacy. We hypothesize that concentrating the dose (by increasing the number of sessions per day and decreasing the intervals between sessions) may enhance efficacy up to a threshold, beyond which administering additional sessions may not yield further benefit or even reduce therapeutic benefit.

To test this hypothesis, we explored in an animal model the efficacy of three differently concentrated 10Hz rTMS protocols delivering the same dose (8 sessions; 1,600 pulses/session), but differing in how densely sessions were scheduled (in terms of number of sessions per day and intersession intervals): (i) a standard protocol (St-rTMS), matching human standard protocols (1 session/day, 8 consecutive days, overnight gaps [5]); (ii) an accelerated protocol (a-rTMS), replicating human accelerated protocols (4 sessions/day, 2 consecutive days, 55-min within-day intersession interval, single overnight gap [6]); (iii) a super-accelerated protocol (sA-rTMS) to assess potential limits in dose temporal distribution (8 sessions in 1 day, 15-min within-day intersession interval, no overnight gap). Each 13-min session comprised 40 trains (40 pulses/4s per train) separated by 16-s inter-train intervals. rTMS was delivered using a MagPro-R30 with a rodent-optimized coil for improved focality, targeting the prefrontal cortex bilaterally. The experimental timeline, with a schematic representation of the stimulation sessions, is presented in Fig. 1a.

Experiments were performed in a validated animal model (Wistar-Kyoto rats exposed to chronic stress), known to exhibit resistance to antidepressants but to respond to deep brain stimulation [7] and ketamine administration [8], thus mirroring key features of TRD clinical picture. Each protocol included a sham group exposed to the same procedure and acoustic effects. Animals were allocated to three independent cohorts, each receiving a single rTMS protocol (St-rTMS n = 34, a-rTMS n = 30, sA-rTMS n = 24); within each cohort, treatment (active/sham) was the sole between-subject factor.

Behavioral tests investigating anxiety and conflict-based motivation (novelty-suppressed feeding test, NSFT), amotivation/apathy (splash test), behavioral despair/helplessness (forced-swim test, FST), anxiety and general locomotor activity (open-field test, OFT) were conducted shortly after the end of the stimulation period. Study design, test procedures and statistical analyses are fully detailed in the supplementary material.

In the splash test, all protocols elicited an anti-apathetic and pro-motivational effect as measured by increased self-grooming behavior compared to the respective sham group (St-rTMS: $\chi^2_1 = 4.51$, $p = 0.03$, Fig. 1c; a-rTMS: $F_{1,28} = 3.86$, $p = 0.05$; Fig. 1f; sA-rTMS: $F_{1,22} = 4.93$, $p = 0.04$ Fig. 1i). In the FST, however, we observed a decrease in helplessness-like behavior only following a-rTMS ($F_{1,28} = 4.60$, $p = 0.04$, Fig. 1g), but not St-rTMS ($F_{1,32} = 0.02$, $p = 0.88$, Fig. 1d) or sA-rTMS ($F_{1,22} = 0.46$, $p = 0.50$, Fig. 1j) as measured by the latency to start floating.

In the NSFT, St-rTMS and a-rTMS, not sA-rTMS, elicited an anxiolytic-like and pro-motivational effect as measured by the shorter latency to approach the food in the arena center (St-rTMS: $F_{1,32} = 5.12$, $p = 0.03$, Fig. 1b; a-rTMS: $F_{1,28} = 5.13$, $p = 0.03$, Fig. 1e; sA-rTMS: $F_{1,22} = 0.25$, $p = 0.62$, Fig. 1h). No effect was observed in the home-cage, confirming changes were not hunger-driven (see supplementary material).

Importantly, an increase in anxiety-like behavior was observed following sA-rTMS in the OFT, as measured by the decreased amount of time spent in the center ($F_{1,22} = 4.42$, $p = 0.05$, eFig. 2e), while no changes were observed following St-rTMS ($F_{1,32} = 1.14$, $p = 0.29$, eFig. 2a) or a-rTMS ($F_{1,28} = 0.08$, $p = 0.78$, eFig. 2c). None of the rTMS protocols affected locomotor activity in the OFT (respectively, $F_{1,32} = 0.05$, $p = 0.82$, eFig. 2b; $F_{1,28} = 0.01$, $p = 0.92$, eFig. 2d; $F_{1,22} = 2.41$, $p = 0.13$; eFig. 2f).

To provide a comparative index of treatment efficacy, we calculated Cohen's d for the main parameter of each behavioral test in each stimulation group (see supplementary material). These values are shown as individual grey dots in Fig. 1k. For each group, the three effect sizes were then averaged to yield a single composite value (blue dots). To aid visualization, we fit a quadratic curve to group means (red line), showing an inverted-U trend; this is descriptive, not a formal model (Fig. 1k).

Our findings revealed that even though the three protocols delivered an equal total number of pulses, a-rTMS produced the most robust antidepressant- and anxiolytic-like effect, while the sA-rTMS failed to confer noticeable benefits, producing instead an anxiogenic-like phenotype. Nonetheless, all protocols reduced the apathy-like state that characterizes the model, possibly indicating that the neural circuits

https://doi.org/10.1016/j.brs.2025.11.003
Received 21 August 2025; Received in revised form 21 October 2025; Accepted 3 November 2025
Available online 6 November 2025

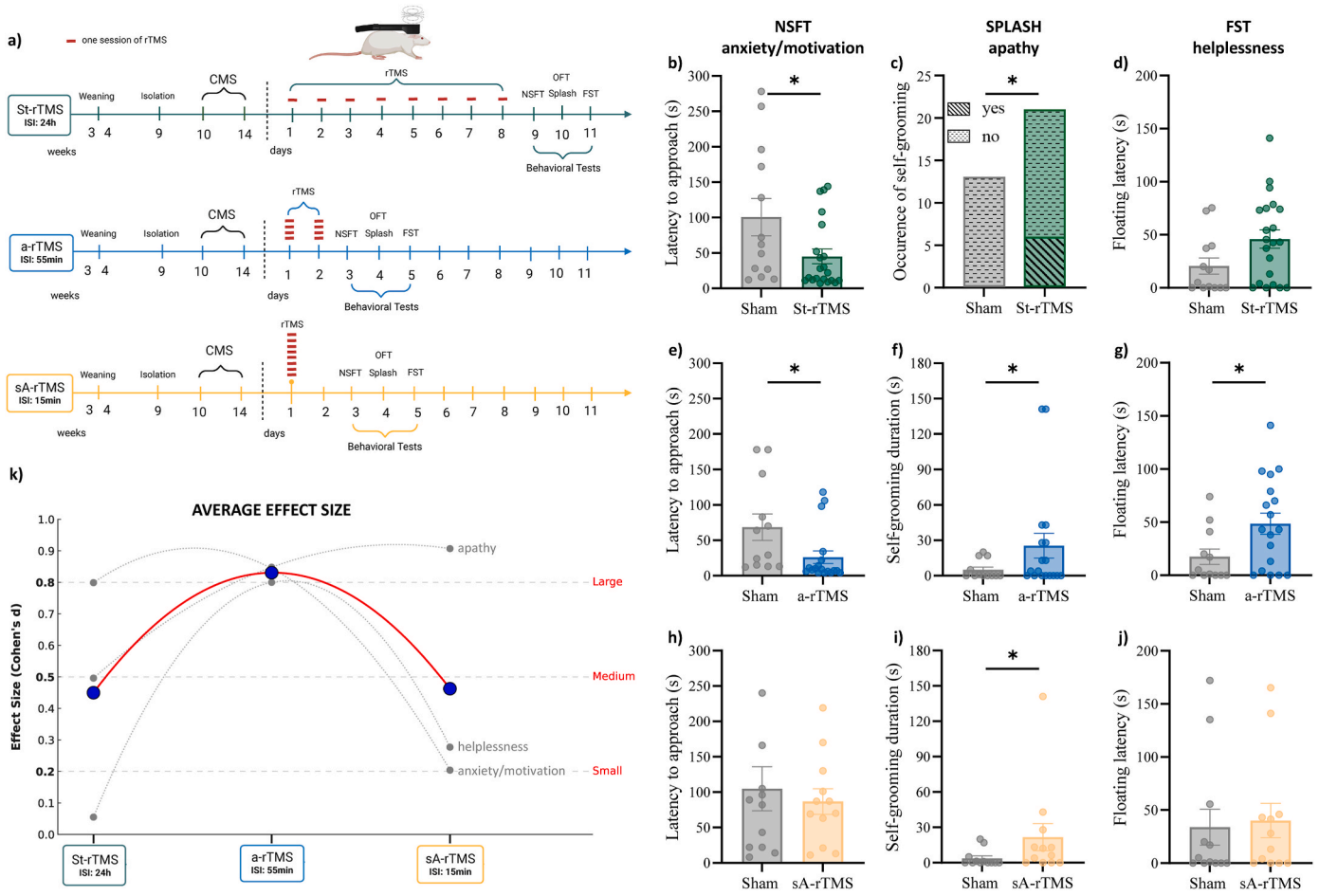

Fig. 1. a) Timeline of experimental procedures (created with BioRender.com); **b)** Latency to approach the food in the NSFT, **c)** occurrence of self-grooming in the splash test, and **d)** floating latency in the FST following St-rTMS (sham n = 13, active n = 21); **e)** Latency to approach the food in the NSFT, **f)** self-grooming duration in the splash test, and **g)** floating latency in the FST following a-rTMS (sham n = 12, active n = 18); **h)** Latency to approach the food in the NSFT, **i)** self-grooming duration in the splash test, and **j)** floating latency in the FST following sA-rTMS (sham n = 12, active n = 12); **k)** Individual effect sizes (Cohen's d) for each behavioral test across the three stimulation groups are shown in grey; group means are indicated in blue. The red line reflects a second-order polynomial fit to the group means, intended to illustrate a potential non-linear (inverted U-shaped) relationship between intersession interval and rTMS efficacy. This curve is included for visualization purposes only and does not represent a formal statistical model. **Abbreviations.** rTMS: repeated transcranial magnetic stimulation; St-rTMS: standard rTMS; a-rTMS: accelerated rTMS; sA-rTMS = super-accelerated rTMS; ISI: intersession interval; CMS: chronic mild stress; NSFT: novelty-suppressed feeding test; OFT = open-field test; FST = forced-swim test. *p ≤ 0.05.

underlying motivational drive are less sensitive to the temporal distribution of stimulation than those mediating affective or anxiety-related responses. General locomotor activity remained unaffected, confirming that behavioral effects were not secondary to motor changes.

Our results highlight the critical role of session density in determining the behavioral efficacy of rTMS. While delivering the same total dose, only a-rTMS, and to a lesser extent St-rTMS—characterized by a more distributed temporal structure—elicited beneficial effects across key domains relevant to TRD. In contrast, the excessively condensed sA-rTMS appeared ineffective or even detrimental, suggesting that overly rapid stimulation delivery may disrupt rather than support adaptive neuroplastic changes.

This discrepancy might reflect distinct neurobiological consequences driven by the temporal dynamics of stimulation. Standard and accelerated protocols may allow sufficient time between sessions to engage synaptic consolidation, homeostatic plasticity, and network-level reorganization—crucial mechanisms for behavioral changes [9]. In contrast, excessively compressed protocols may not, potentially leading to saturation or maladaptive plasticity. Rapid-fire stimulation might also disrupt local excitation-inhibition balance or desynchronize large-scale networks, especially within cortico-limbic circuits implicated in mood regulation. Moreover, excessive metabolic or neurochemical demands imposed by super-accelerated delivery could activate stress-related pathways or impair glial support functions, counteracting therapeutic effects. These results align with evidence that ~40–50min inter-stimulation intervals optimize LTP induction, whereas shorter intervals do not [10]. Consistently, our recent meta-regression of accelerated rTMS trials in depression showed a significant dose–response relationship in clinical outcomes and indicated that longer inter-session intervals (≥50min) were associated with greater treatment effectiveness [11].

In addition, rTMS protocols, by delivering temporally patterned perturbations, may interact with ongoing intrinsic dynamics in complex ways, influencing network stability and plasticity. The observed inverted U-shaped relationship between stimulation timing and efficacy might reflect optimal alignment with the brain's intrinsic variability, where certain intersession intervals effectively harness stochastic resonance to promote adaptive neuroplastic changes, whereas overly rapid or excessively spaced stimulation disrupts this balance. Using a rodent-optimized coil improved focality, yet spatial selectivity is still below that of modern human figure-8 coils; this concerns anatomical precision rather than the temporal dosing structure and does not alter the inferred inverted-U.

In conclusion, the present findings demonstrate that stimulation

timing critically contribute to determine rTMS efficacy and suggest an inverted U-shaped relationship between dose distribution and therapeutic outcomes. a-rTMS with optimally spaced intersession intervals appeared more effective, whereas both overly condensed and excessively prolonged intervals were associated with reduced therapeutic benefit. This interpretation aligns with prior proposals that multiple rTMS parameters follow an inverted-U function, with peak efficacy at intermediate values [12]. Future studies will be needed to investigate the underlying neurobiology across cellular, circuit, and systems levels. These findings could contribute to the optimization of rTMS protocols, ultimately increasing their efficacy in the treatment of clinical depression.

CRediT authorship contribution statement

Carola Cerri: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Marta Boffa:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Antonio Inserra:** Writing – original draft, Visualization. **Sara Spalletti:** Methodology, Investigation, Data curation. **Martina Bertone:** Methodology, Investigation. **Domenico Voso:** Writing – review & editing. **Roberto Guidotti:** Writing – review & editing. **Vittorio Pizzella:** Writing – review & editing, Conceptualization. **Laura Marzetti:** Writing – review & editing, Conceptualization. **Giorgio Di Lorenzo:** Writing – review & editing, Conceptualization. **Luisa De Risio:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Giovanni Martinotti:** Writing – review & editing, Conceptualization. **Mauro Pettorruso:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Francesca Zoratto:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Ethics approval

All experiments were approved by the Ministry of Health (authorization no. 8/2021-PR, protocol D9997.123) in full compliance with Legislative Decree 26/2014 (implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes).

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Funding

Italian Ministry of Health under the “Ricerca Finalizzata, Young Researchers grant” (to MP, LDR and FZ; grant code GR-2019-12370173).

Declaration of competing interest

The authors declare no competing interests.

Acknowledgments

The authors wish to thank Luigia Cancemi, Andrea Martinelli, Paolo Frassanito, and Flavio Torriani for their valuable assistance with animal care.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2025.11.003.

References

[1] Lefaucheur JP, Colzi C, Hollander E, Pampaloni I, Van Ameringen M, Baeken C, Fusar-Poli P, Arango C, Fontenelle LF, Batail JM, Brunoni AR, Nicolini H, Haffen E, Soriano-Mas C, Rodriguez CI, Dell'Osso BM, Vieta E, Sauvaget A, Szekely D, Mayer-Linderberg A, Denys D, Stein DJ, Drapier D, Voon V, Pallanti S. Comparison between accelerated and standard or sham rTMS in the treatment of depression: a systematic review. Neurosci Biobehav Rev 2025;173:106140. https://doi.org/10.1016/j.neubiorev.2025.106140.

[2] Modirrousta M, Meek BP, Wikstrom SL. Efficacy of twice-daily vs once-daily sessions of repetitive transcranial magnetic stimulation in the treatment of major depressive disorder: a retrospective study. Neuropsychiatric Dis Treat 2018;14:309–16. https://doi.org/10.2147/NDT.S151841.

[3] Caulfield KA, Fleischmann HH, George MS, McTeague LM. A transdiagnostic review of safety, efficacy, and parameter space in accelerated transcranial magnetic stimulation. J Psychiatr Res 2022;152:384–96. https://doi.org/10.1016/j.jpsychires.2022.06.038.

[4] Hsu TW, Yeh TC, Kao YC, Thompson T, Brunoni AR, Carvalho AF, Hsu CW, Tu YK, Liang CS. The dose-effect relationship of six stimulation parameters with rTMS over left DLPFC on treatment-resistant depression: a systematic review and meta-analysis. Neurosci Biobehav Rev 2024;162:105704. https://doi.org/10.1016/j.neubiorev.2024.105704.

[5] De Risio L, Borgi M, Pettorruso M, Miuli A, Ottomana AM, Sociali A, Martinotti G, Nicolò G, Macrì S, di Giannantonio M, Zoratto F. Recovering from depression with repetitive transcranial magnetic stimulation (rTMS): a systematic review and meta-analysis of preclinical studies. Transl Psychiatry 2020;10(1):393. https://doi.org/10.1038/s41398-020-01055-2.

[6] Pettorruso M, d'Andrea G, Di Carlo F, De Risio L, Zoratto F, Miuli A, Benatti B, Vismara M, Pompili E, Nicolò G, Niolu C, Siracusano A, Sensi SS, Dell'Osso B, Di Lorenzo G, Martinotti G, ReModula Study Group. Comparing fast-acting interventions for treatment-resistant depression: an explorative study of accelerated HF-rTMS versus intranasal esketamine. Brain Stimul 2023;16(4):1041–3. https://doi.org/10.1016/j.brs.2023.06.003.

[7] Papp M, Gruca P, Lason M, Niemczyk M, Willner P. The role of prefrontal cortex dopamine D2 and D3 receptors in the mechanism of action of venlafaxine and deep brain stimulation in animal models of treatment-responsive and treatment-resistant depression. J Psychopharmacol 2019;33(6):748–56. https://doi.org/10.1177/0269881119827889.

[8] Willner P, Gruca P, Lason M, Tota-Glowczyk K, Litwa E, Niemczyk M, Papp M. Validation of chronic mild stress in the Wistar-Kyoto rat as an animal model of treatment-resistant depression. Behav Pharmacol 2019;30:239–50. https://doi.org/10.1097/FBP.0000000000000431.

[9] Makkinayeri S, Guidotti R, Basti A, Woolrich MW, Gohil C, Pettorruso M, Ermolova M, Ilmoniemi RJ, Ziemann U, Romani GL, Pizzella V, Marzetti L. Investigating brain network dynamics in state-dependent stimulation: a concurrent electroencephalography and transcranial magnetic stimulation study using hidden Markov models. Brain Stimul 2025;18(3):800–9. https://doi.org/10.1016/j.brs.2025.03.020.

[10] Lynch G, Kramár EA, Babayan AH, Rumbaugh G, Gall CM. Differences between synaptic plasticity thresholds result in new timing rules for maximizing long-term potentiation. Neuropharmacology 2013;64(1):27–36. https://doi.org/10.1016/j.neuropharm.2012.07.006.

[11] Pettorruso M, Borgi M, Padula LP, Miuli A, Benatti B, Guidotti R, Marzetti L, Baeken C, Dell'Osso B, Di Lorenzo G, Zoratto F, Martinotti G. Meta-regression insights for optimizing accelerated neuromodulation protocols in major depression. J Psychiatr Res 2025. in press.

[12] Caulfield KA, Brown JC. The problem and potential of TMS' infinite parameter space: a targeted review and road map forward. Front Psychiatr 2022;13:867091. https://doi.org/10.3389/fpsyt.2022.867091.

Carola Cerri[a,1], Marta Boffa[a,b,1], Antonio Inserra[b], Sara Spalletti[a], Martina Bertone[a], Domenico Voso[b], Roberto Guidotti[b,c], Vittorio Pizzella[b,c], Laura Marzetti[c,d], Giorgio Di Lorenzo[e,f], Luisa De Risio[g], Giovanni Martinotti[b,c,h,i], Mauro Pettorruso[b,c,h,2,*], Francesca Zoratto[a,2]

[a] *Centre for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy*

[b] *Department of Neuroscience, Imaging and Clinical Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy*

[c] *Institute for Advanced Biomedical Technologies (ITAB), "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy*

[d] *Department of Engineering and Geology, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy*

[e] *Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy*

[f] *IRCCS Fondazione Santa Lucia, Rome, Italy*

[g] *Department of Mental Health and Addiction, ASL Roma 5, Rome, Italy*

[h] *Department of Mental Health, ASL 2 Lanciano-Vasto-Chieti, Chieti, Italy*

[i] *Psychopharmacology, Drug Misuse and Novel Psychoactive Substances Research Unit, School of Life and Medical Sciences, University of Hertfordshire, Hatfield, United Kingdom*

[*] Corresponding author. Department of Neuroscience, Imaging and Clinical Sciences, "G. d'Annunzio" University of Chieti-Pescara, Via Luigi Polacchi 11, 66100 Chieti, Italy.
E-mail address: mauro.pettorruso@unich.it (M. Pettorruso).

[1] These authors contributed equally (co-first authors).

[2] These authors contributed equally (co-last authors).