NEUROPHYSIOLOGY

Elevating cerebellar theta oscillations boosts noninvasively induced motor plasticity

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Matching brain stimulation to the brain's natural rhythms can drive plasticity, yet this principle has rarely been tested in humans. We targeted the cerebellum, a key hub for motor coordination and learning, using a rhythm-tuned protocol that pairs theta-frequency transcranial alternating current stimulation with intermittent theta-burst stimulation to engage plasticity of cerebello-cortical circuits. In young healthy adults, this pairing enhanced fine motor control and hand dexterity, with gains closely tracking physiological markers of cerebellar-driven plasticity. Applying the same approach in chronic stroke survivors yielded parallel behavioral and neural gains, demonstrating preserved rhythm-plasticity coupling despite injury. Control experiments confirmed both frequency specificity and site specificity, underscoring the mechanistic precision of the intervention. By linking theta-frequency cerebellar stimulation to circuit-level and functional outcomes, these findings establish a biologically grounded framework for targeted neurorehabilitation. Rhythm-specific cerebellar stimulation provides a scalable strategy for enhancing plasticity and improving motor function across movement disorders and motor impairments.

INTRODUCTION

Frequency-tuned neuromodulation is a powerful yet underexplored strategy to enhance plasticity in subcortical brain circuits. The cerebellum, long recognized for its role in motor control and learning, is emerging as a key modulator of experience-dependent plasticity, but the underlying mechanisms remain poorly understood. One candidate is intrinsic theta-band (4 to 8 Hz) activity, which organizes input-output transformations in cerebellar microcircuits and drives synaptic plasticity via spike-timing and *N*-methyl-D-aspartate/nitric oxide (NMDA/NO)–dependent mechanisms (*1*–*3*).

Although theoretical models highlight the importance of cerebellar theta dynamics, it remains unknown whether externally entraining these rhythms can modulate plasticity and behavior in humans. Aligning neuromodulation to intrinsic cerebellar activity offers a biologically grounded means of engaging circuit-specific plasticity (*4*–*7*), an especially promising approach in stroke, where cortical plasticity is often impaired but cerebello-cortical pathways remain structurally intact and functionally responsive (*8*–*10*).

Cerebellar intermittent theta-burst stimulation (iTBS) has shown that the cerebellum is responsive to noninvasive neuromodulation and may facilitate motor recovery (*8*). However, iTBS alone does not directly target ongoing oscillatory activity. To address this, we introduce a dual-modality, rhythm-tuned protocol that pairs concurrent 5-Hz transcranial alternating current stimulation (theta tACS) with cerebellar iTBS. Grounded in models of cerebellar resonance and circuit excitability (*11*–*13*), this approach is designed to target theta-band dynamics and amplify downstream motor plasticity ordinarily engaged by iTBS. In patients with stroke, stimulation was delivered to the cerebellar hemisphere contralateral to the cortical lesion to align with crossed cerebello-thalamo-cortical projections through which the cerebellum modulates motor cortex excitability.

Across experiments in young healthy adults and individuals with chronic stroke, we demonstrate that theta-frequency cerebellar stimulation produces robust, frequency-specific enhancements in physiological plasticity and fine motor function. These findings establish cerebellar theta entrainment as a circuit-informed, translationally scalable strategy for restoring function under conditions characterized by impaired cortical plasticity.

RESULTS

We first tested whether combining cerebellar theta tACS with iTBS enhances motor plasticity in healthy individuals. In experiment 1, 14 young adults received right cerebellar-targeted iTBS paired with concurrent tACS delivered at sham, theta ($\theta = 5$ Hz), or gamma ($\gamma = 50$ Hz) frequency (referred to as sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS; Fig. 1, A to C). Biophysical modeling confirmed focal targeting of the lateral cerebellum, with peak intensities in the range of 35 to 45 V/m, supporting the precision and efficacy of our targeting approach.

Corticospinal excitability, indexed by motor-evoked potentials (MEPs) recorded from the right first dorsal interosseous (FDI) following single-pulse transcranial magnetic stimulation (TMS) of the left primary motor cortex (M1), served as the physiological proxy for cerebellar-motor plasticity. A repeated-measures analysis of variance (RM-ANOVA) with SESSION (sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS) and TIME (T0, T15, and T30) revealed a significant main effect of SESSION [$F(2,38) = 7.070$, $P = 0.002$, and $\eta^2_p = 0.270$], indicating robust differences in plasticity across stimulation conditions. No significant main effect of TIME or SESSION × TIME interaction was observed, indicating that the modulation was condition specific and stable over time. Holm-corrected tests

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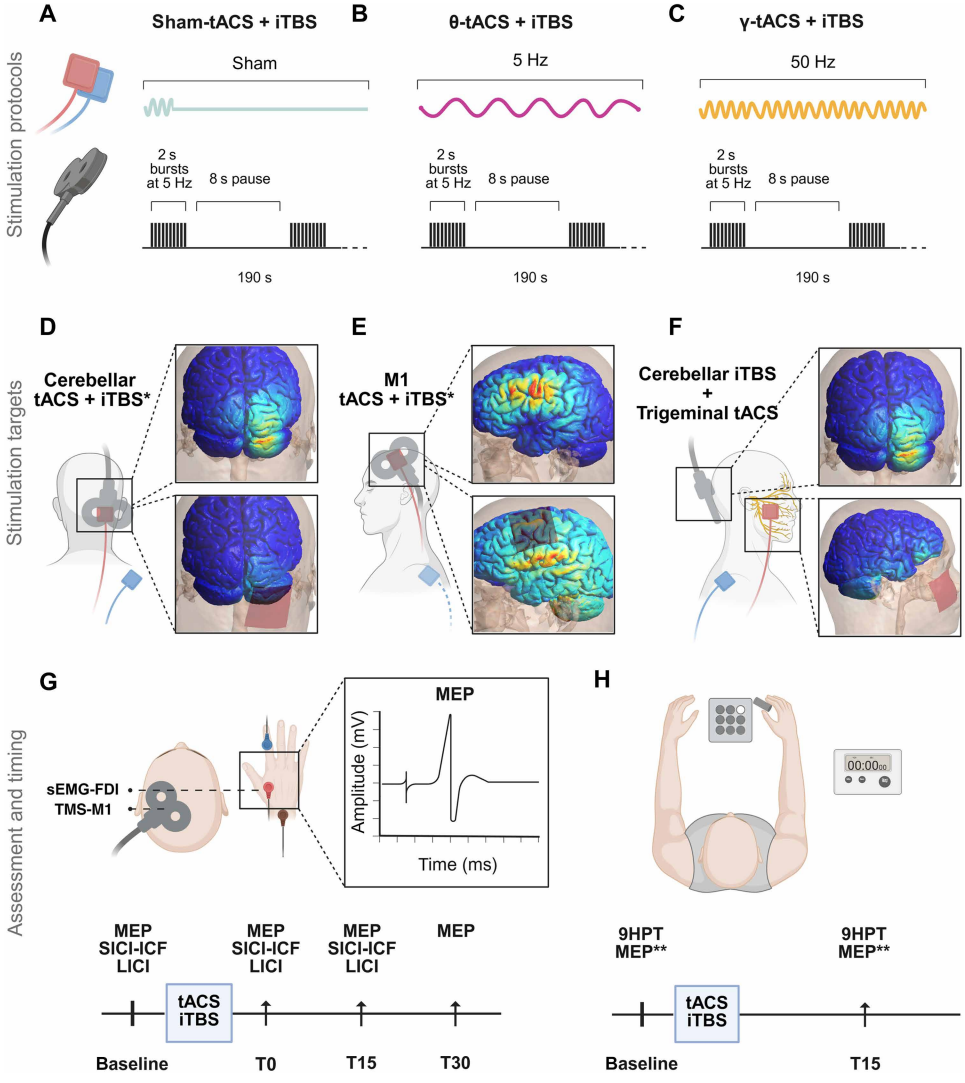

Fig. 1. Experimental conditions and protocol overview. (**A** to **C**) tACS (2 mA) was paired with cerebellar iTBS (600 pulses delivered over 190 s in 2-s trains of three pulses at 50 Hz, repeated at 5 Hz, at 80% AMT). Three stimulation conditions were used: (A) sham-tACS, (B) θtACS (5 Hz), and (C) γtACS (50 Hz). (**D** to **F**) Stimulation sites included the (D) lateral cerebellum (3 cm lateral and 1 cm inferior to the inion) and (E) the primary motor cortex (M1). (F) In the sensory-control condition, θtACS was applied over the maxillary branch of the trigeminal nerve, whereas iTBS was delivered to the cerebellum, matching somatosensory input and electrode-coil spacing while minimizing cerebellar current flow. (**G**) Neurophysiological outcomes included single-pulse MEPs to assess corticospinal excitability and paired-pulse TMS protocols to quantify short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and long-interval intracortical inhibition (LICI). Measures were obtained at baseline and at 0 min (T0), 15 min (T15), and 30 min (T30) after stimulation. (**H**) Manual dexterity was assessed using the 9HPT, administered before and 15 min after stimulation. *In patients with stroke, stimulation was applied to the cerebellar hemisphere contralateral to the lesion and to the ipsilesional M1. **MEP measurements during 9HPT testing were collected in healthy participants only. Created in BioRender. Martino Cinnera, A. (2026) https://BioRender.com/m0kw2ak.

confirmed that θtACS + iTBS produced significantly greater MEP facilitation than sham-tACS + iTBS [mean difference = 0.50, 95% confidence interval (CI): 0.15 to 0.84, $P = 0.004$, and Cohen's $d = 0.83$; Fig. 2A], whereas γtACS + iTBS did not produce significant modulation (Fig. 2B). A distribution-based heatmap responder analysis (fig. S1) showed that θtACS + iTBS elicited the highest proportion of responders (13/20), demonstrating strong frequency specificity at both group and individual levels. Together, these findings suggest that theta-frequency cerebellar stimulation primes cerebello-cortical networks for enhanced plastic responsiveness to iTBS.

To further characterize the physiological effects of stimulation, we assessed intracortical inhibition and facilitation using standard paired-pulse TMS protocols: short-interval intracortical inhibition (SICI; 2 ms), intracortical facilitation (ICF; 15 ms), and long-interval intracortical inhibition (LICI; 100 ms). An RM-ANOVA on SICI with SESSION (sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS) and TIME (baseline, T0, and T15) revealed no main effect of SESSION [$F(2,38) = 0.15$, $P = 0.864$, and $\eta^2_p = 0.008$] and no main effect of TIME [$F(2,38) = 0.71$, $P = 0.497$, and $\eta^2_p = 0.036$]. However, a significant SESSION × TIME interaction emerged [$F(4,76) = 3.29$, $P = 0.015$, and $\eta^2_p = 0.147$], indicating that changes in SICI depended on stimulation condition.

Follow-up one-way ANOVAs across TIME showed a significant TIME effect for θtACS + iTBS [$F(2,38) = 4.76$; $P = 0.014$]. Post hoc

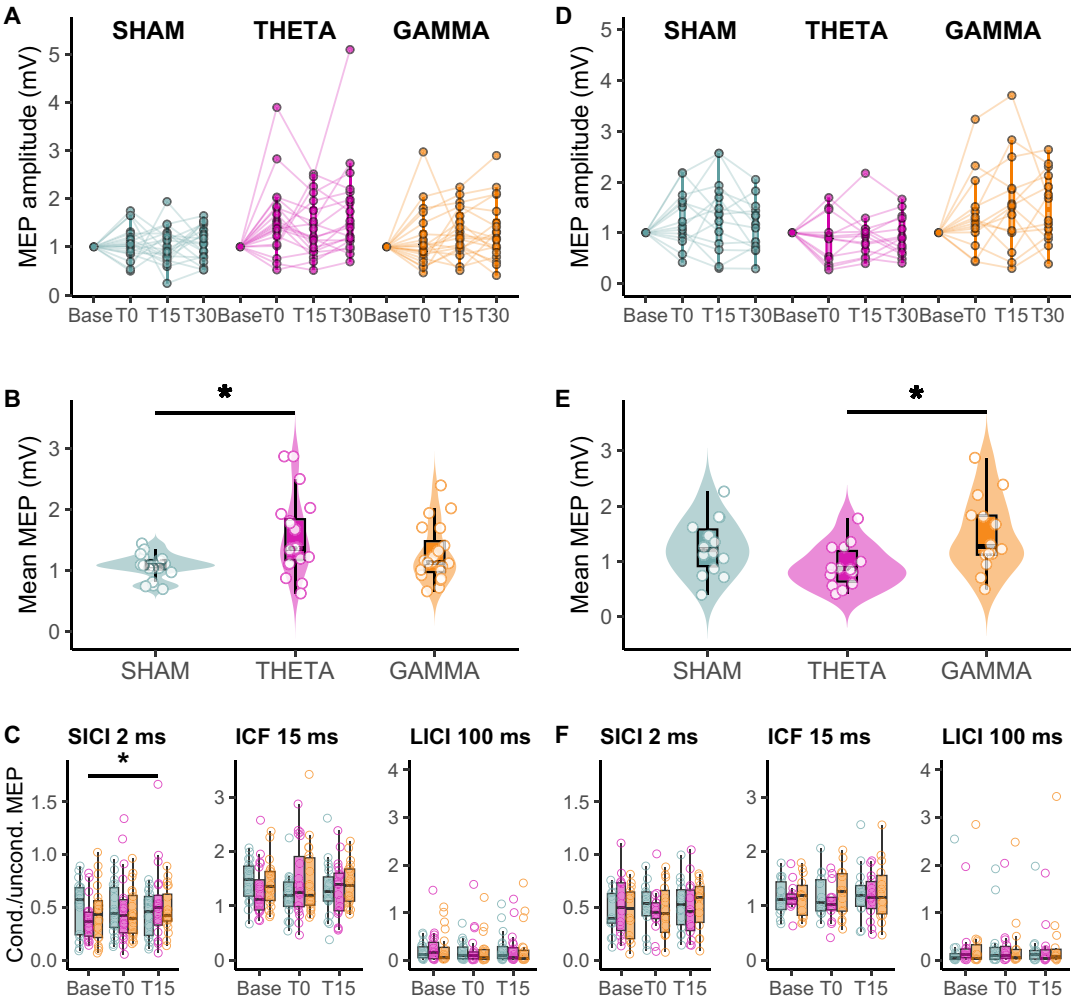

Fig. 2. Cerebellar θtACS + iTBS selectively enhances corticospinal excitability via frequency-specific plasticity. (**A**) Time course of normalized MEP amplitudes following cerebellar stimulation. θtACS + iTBS induced a sustained increase in corticospinal excitability lasting up to 30 min postintervention, whereas γtACS + iTBS and sham-tACS + iTBS produced no significant modulation. (**B**) Group-level comparison of mean MEP amplitudes. θtACS + iTBS produced significantly greater facilitation than sham-tACS + iTBS ($P = 0.004$), demonstrating frequency-specific enhancement. Distributions are shown using half-violins overlaid with box plots to illustrate variability and central tendency. (**C**) Intracortical excitability assessed using paired-pulse TMS. θtACS + iTBS significantly reduced SICI (2 ms) at T15 relative to baseline, consistent with diminished $GABA_A$-mediated inhibition. No significant effects were observed for ICF (15 ms) or LICI (100 ms). (**D**) Normalized MEP amplitudes over time following left M1 stimulation. γtACS + iTBS increased corticospinal excitability, whereas θtACS + iTBS did not enhance and, in some cases, attenuated the typical facilitatory response. (**E**) Group-level comparison following M1 stimulation. γtACS + iTBS elicited significantly greater MEP amplitudes than θtACS + iTBS ($P < 0.05$); neither condition differed from sham. (**F**) Paired-pulse measures following M1 stimulation revealed no significant effects of SESSION or TIME on SICI, CF, or LICI.

tests showed reduced SICI at T15 relative to baseline [$t(19) = -3.01$; $P = 0.021$], consistent with a delayed disinhibitory effect (Fig. 2C). No significant differences were detected between baseline and T0 ($P = 0.078$) or between T0 and T15 ($P = 0.394$). In contrast, γtACS + iTBS [$F(2,38) = 0.27$; $P = 0.763$] and sham-tACS + iTBS [$F(2,38) = 1.45$; $P = 0.248$] showed no TIME effects. No significant effects were found for ICF or LICI under any condition (all $P > 0.05$), nor were significant time effects observed in the sham or gamma sessions (Fig. 2C). The selective reduction of SICI following θtACS + iTBS represents the only intracortical measure with condition-specific temporal modulation, aligning with the enhanced corticospinal facilitation observed in MEPs.

To evaluate the anatomical and frequency specificity of the stimulation effects, we applied the same dual-stimulation protocol (tACS + iTBS) over the M1 in experiment 2. In contrast to cerebellar stimulation, θtACS + iTBS over M1 did not enhance corticospinal excitability and, in some cases, appeared to blunt the typical facilitatory response to iTBS (Fig. 2D). An RM-ANOVA with SESSION (sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS) as a within-subject factor revealed a significant main effect of SESSION [$F(2,26) = 4.39$, $P = 0.023$, and $\eta^2_p = 0.253$] with no main effect of TIME ($P = 0.362$) and no SESSION × TIME interaction ($P = 0.568$). Holm-corrected post hoc tests indicated significantly higher MEP amplitudes during γtACS + iTBS compared to θtACS + iTBS ($P = 0.047$), whereas neither active condition differed significantly from sham-tACS + iTBS ($P > 0.08$; Fig. 2E).

No significant main effects or interactions were observed for SICI, ICF, or LICI across sessions or time points (all $P > 0.05$; Fig. 2F),

further underscoring the anatomical specificity of cerebellar theta stimulation in modulating intracortical excitability. A pooled cross-site analysis comparing cerebellar and M1 stimulation confirmed a significant CONDITION (sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS) × SITE (cerebellum and M1) interaction, driven by the selective facilitation observed only during cerebellar θtACS + iTBS (see the Supplementary Materials).

To determine whether the effects of cerebellar θtACS + iTBS were driven by peripheral costimulation rather than cerebellar engagement, we conducted experiment 3 in 10 healthy participants. Under this control condition, θtACS was delivered over a trigeminal-afferent montage on the cheek, whereas iTBS was applied to the right cerebellum (Fig. 1F), thereby matching somatosensory input and coil-electrode spacing but minimizing cerebellar current flow. Collapsing across poststimulation time points, cerebellar θtACS + iTBS produced significantly greater MEP facilitation than trigeminal θtACS + iTBS [mean difference = 0.57 ± 0.21, $t(9) = 2.69$, $P = 0.025$, and Cohen's $d = 0.98$] (Fig. 3, A and B). These results demonstrate that the enhancement depends on cerebellar current flow and cannot be attributed to peripheral trigeminal or cutaneous costimulation alone.

We next asked whether these physiological changes translated into behavioral gains. In experiment 4, participants completed the Nine-Hole Peg Test (9HPT), a validated measure of fine motor control, before and after cerebellar θtACS + iTBS or sham-tACS + iTBS (Fig. 4A). On the basis of our a priori hypothesis that theta-frequency stimulation would enhance motor performance, we tested whether improvement (Δ = Pre − Post) was greater following θtACS + iTBS than sham. A directional paired-samples t test confirmed significantly greater improvement after θtACS + iTBS [$t(17) = 1.93$, $P = 0.035$, one-tailed, and Cohen's $d = 0.46$] (Fig. 4, A and B). On average, participants improved 0.46 s more after θtACS + iTBS than sham-tACS + iTBS (95% CI: −0.03 to 0.95). Across individuals, Δθ ranged from −0.58 to 3.28 s (mean = 1.06; SD = 0.91), whereas Δsham ranged from −1.45 to 1.66 s (mean = 0.60; SD = 0.72).

To characterize individual variability, we classified participants as responders when their improvement after θtACS + iTBS exceeded their own improvement after sham-tACS + iTBS, which alone can enhance motor performance (*14*). By this within-subject criterion, 10 of 18 participants (56%) showed additional improvement with θtACS + iTBS. These findings demonstrate that cerebellar theta-aligned stimulation enhances fine motor performance in young healthy adults, albeit with notable interindividual variability.

In the same individuals, an RM-ANOVA revealed a significant main effect of TIME on MEP amplitude [$F(1,17) = 15.340$, $P = 0.001$, and $\eta^2_p = 0.474$] but no significant SESSION or TIME × SESSION interaction effects (Fig. 4C), replicating the core physiological findings from experiment 1 in an independent cohort. Only under the θtACS + iTBS condition was the change in MEP amplitude significantly correlated with behavioral improvement ($r = 0.559$; $P = 0.024$; Fig. 4D), whereas no such relationship was observed under sham-tACS + iTBS. This brain-behavior link provides compelling evidence that cerebellar theta-aligned stimulation engages physiologically and functionally relevant plasticity mechanisms.

Building on these findings, we tested whether theta-frequency cerebellar stimulation could re-engage spared plasticity pathways and promote recovery in individuals with chronic stroke. In experiment 5, a randomized, sham-controlled, crossover study, patients received sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS across three sessions using the same within-subject design as in healthy participants. An RM-ANOVA revealed a significant main effect of

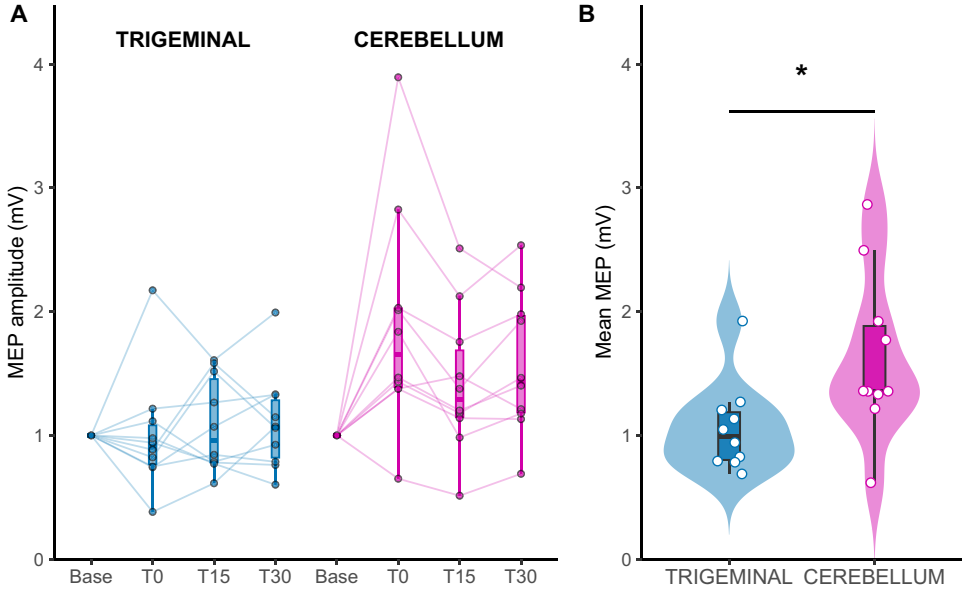

Fig. 3. Cerebellar current flow, and not peripheral trigeminal stimulation, drives the enhancement of corticospinal excitability. (**A**) Time course of normalized MEP amplitudes comparing cerebellar θtACS + iTBS with a trigeminal-site control condition. In both sessions, participants received identical cerebellar iTBS parameters and identical tACS frequency (5 Hz); the only difference was the tACS montage, delivered either over the lateral cerebellum or over the maxillary division (V2) of the trigeminal nerve. Cerebellar θtACS + iTBS produced a larger and more sustained facilitation of corticospinal excitability across all poststimulation time points (T0, T15, and T30), whereas trigeminal-site θtACS did not elicit similar changes. (**B**) Group-level distribution plots (half-violin + box plots) show significantly higher mean MEP amplitudes during cerebellar θtACS + iTBS relative to trigeminal-site θtACS, demonstrating that the excitatory effect depends on cerebellar current flow rather than peripheral cranial nerve or cutaneous costimulation.

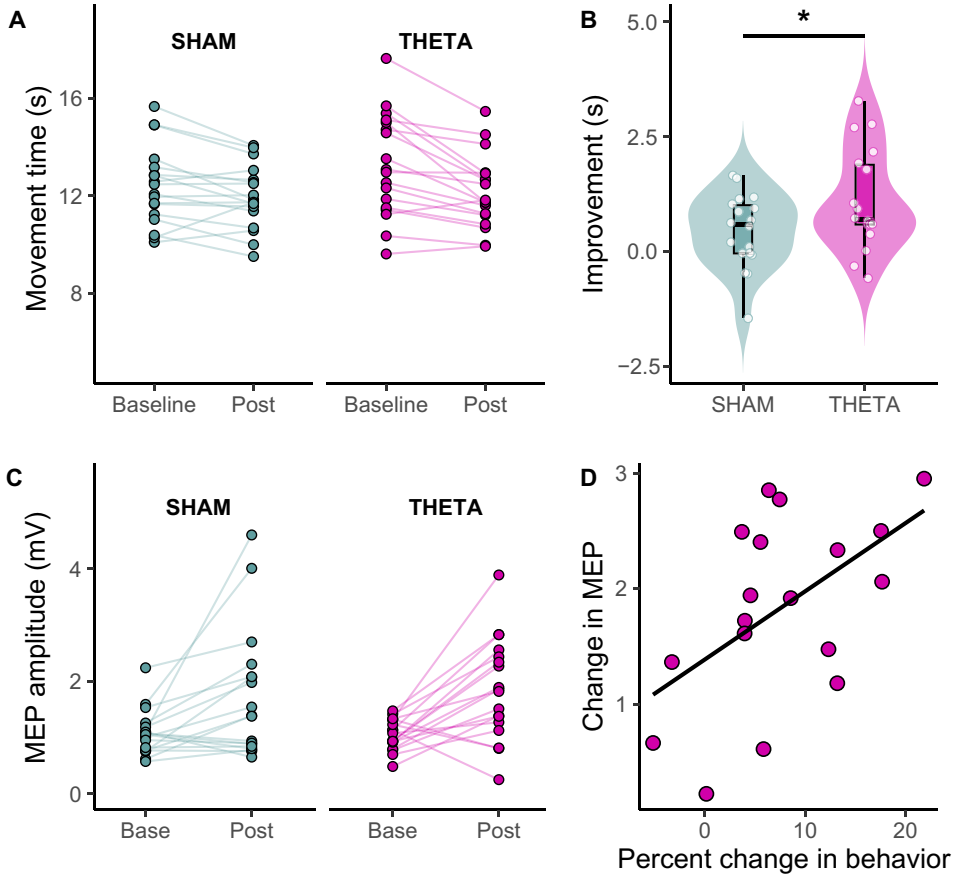

Fig. 4. Cerebellar θtACS + iTBS improves manual dexterity and links physiological plasticity to behavior. (**A**) Experimental timeline for the 9HPT. Participants completed the task immediately before (baseline) and 15 min after stimulation (post). (**B**) Cerebellar θtACS + iTBS produced significantly greater improvement in 9HPT performance compared to sham-tACS + iTBS (paired *t* test, $P = 0.035$, one-tailed), indicating enhanced fine motor function. Violin + box plots display individual variability and central tendency. (**C**) Normalized MEPs increased over time in both sessions, with a significant main effect of TIME but no SESSION or SESSION × TIME interaction, replicating the physiological pattern observed in experiment 1. (**D**) Only under the θtACS + iTBS condition was the change in MEP amplitude significantly correlated with improvement on the 9HPT (Pearson $r = 0.559$, $P = 0.024$), whereas no relationship was present under sham. This selective brain-behavior coupling indicates that cerebellar theta-aligned stimulation engages plasticity mechanisms with functional relevance.

SESSION on corticospinal excitability [$F(2,22) = 4.79$, $P = 0.019$, and $\eta^2_p = 0.303$], indicating differential modulation across stimulation conditions, with no significant effects of TIME ($P = 0.085$) or SESSION × TIME interaction ($P = 0.359$) (Fig. 5A).

Holm-corrected post hoc tests showed that θtACS + iTBS produced significantly greater MEP facilitation than γtACS + iTBS [mean difference = 0.48 ± 0.14, $t(11) = 3.34$, $P = 0.020$, and $d = 0.91$], whereas neither θ nor γ stimulation differed significantly from sham-tACS + iTBS (both $P > 0.21$) (Fig. 5B). These results reinforce the frequency specificity of cerebellar theta stimulation while reflecting the known heterogeneity and relatively strong baseline responsiveness to iTBS in chronic stroke. No significant main effects or interactions were observed for SICI, ICF, or LICI (all $P > 0.05$; Fig. 5C), consistent with reduced intracortical inhibitory and facilitatory modulation in the lesioned hemisphere.

To evaluate clinical relevance in individuals with upper limb impairment due to stroke, experiment 6 assessed fine motor skills performance using the 9HPT in a randomized, sham-controlled, crossover design (Fig. 5D). A directional paired-samples *t* test confirmed that θtACS + iTBS produced greater improvement than sham stimulation [$t(13) = 2.05$, $P = 0.031$, and $d = 0.54$], and a Wilcoxon signed-rank test yielded a consistent result ($P = 0.0075$), demonstrating robustness across analytical approaches (Fig. 5E). On average, θtACS + iTBS produced 1.97 s greater improvement than sham (95% CI [−0.11, 4.06]).

Interindividual variability was expected in chronic stroke: Δ θtACS + iTBS ranged from −0.16 to 19.05 s (mean = 4.34; SD = 5.10), whereas Δ sham-tACS + iTBS ranged from −2.68 to 11.44 s (mean = 2.37; SD = 4.32). Using a within-subject responder criterion (Δθ > Δsham), 10 of 14 patients (71%) showed greater performance gains with θtACS + iTBS, despite iTBS alone being known to enhance motor function in chronic stroke (*8*). These findings demonstrate that most individuals exhibited superior dexterity gains following frequency-matched cerebellar theta stimulation.

Together, these results establish cerebellar θtACS + iTBS as a frequency-specific, circuit-targeted neuromodulation strategy that enhances motor behavior in individuals with chronic stroke. Figure 5F illustrates the anatomical diversity of stroke lesions, showing that benefits were observed even in patients with widespread cortical and subcortical damage. These findings indicate that cerebellar

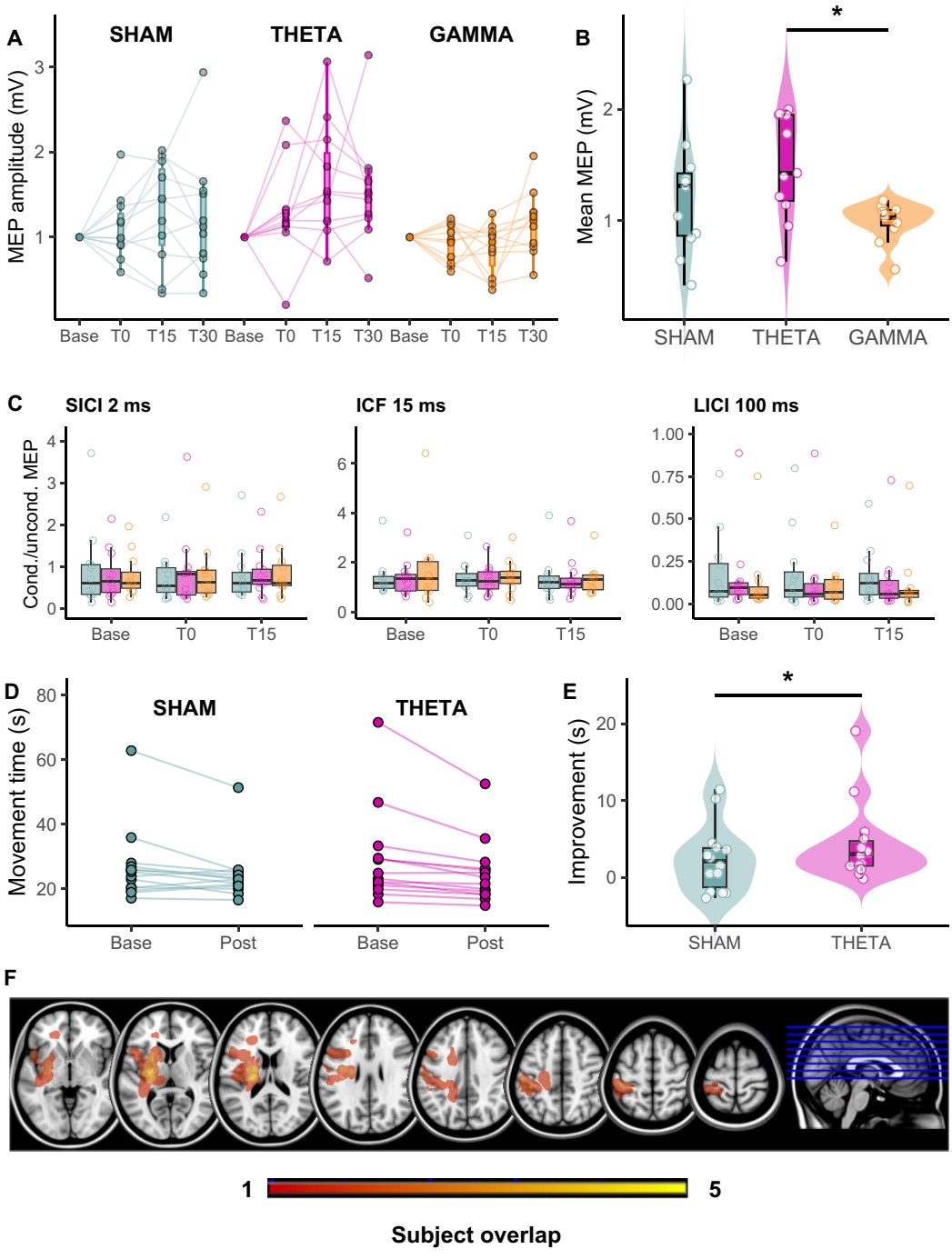

Fig. 5. Cerebellar θtACS + iTBS selectively enhances corticospinal plasticity and manual dexterity in individuals with chronic stroke. (**A**) Time course of normalized MEP amplitudes across stimulation conditions (sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS). An RM-ANOVA revealed a significant main effect of SESSION, indicating the differential modulation of corticospinal excitability. (**B**) Group-level comparisons showed significantly greater MEP facilitation following θtACS + iTBS relative to γtACS + iTBS ($P < 0.05$). Neither active condition differed significantly from sham, consistent with elevated baseline responsiveness to cerebellar iTBS in chronic stroke. Violin/box plots depict distributions and central tendency. (**C**) Intracortical excitability measures showed no significant effects of SESSION or TIME, indicating that stimulation effects were specific to corticospinal output rather than intracortical inhibitory/facilitatory circuits. (**D**) Individual 9HPT trajectories showing movement times before and after stimulation. Most participants demonstrated greater improvement following θtACS + iTBS relative to sham. (**E**) Group-level analysis confirmed significantly faster task completion following θtACS + iTBS ($P < 0.05$), demonstrating enhanced manual dexterity. (**F**) Lesion overlap map for stroke participants ($n = 13$), displayed in MNI space. Colors represent the number of individuals with a lesion at each voxel (overlap count). Lesions encompassed both cortical regions (including primary motor and premotor cortices) and subcortical structures (e.g., internal capsule and basal ganglia), illustrating the anatomical heterogeneity across the patient sample.

theta-aligned stimulation engages physiologically meaningful plasticity and represents a clinically actionable strategy for improving motor function.

DISCUSSION

Theta-band oscillations are central to how the cerebellum orchestrates synaptic plasticity, refines sensorimotor integration, and supports fine motor control (*1*, *3*, *15*, *16*). These intrinsic rhythms provide a powerful temporal scaffold for experience-driven adaptation. Here, we found that cerebellar theta-aligned tACS substantially enhances the induction of cerebello-motor plasticity and improves fine motor behavior in humans. By tuning stimulation to the cerebellum's endogenous frequency, we selectively increased circuit responsiveness and induced durable, functionally meaningful improvements in both healthy individuals and patients with stroke. These findings establish cerebellar theta stimulation as a fine-tuned, circuit-specific strategy for enhancing plasticity in the human motor system.

Our findings align with long-standing theories implicating cerebellar theta activity in spike-timing–dependent plasticity and error-based learning (*4*, *5*, *13*, *14*, *17*). Although iTBS alone produces modest and variable effects (*8*–*10*, *18*), pairing it with θtACS appears to engage cerebellar networks at their endogenous frequency, thereby substantially enhancing plasticity. This synergy increased both the magnitude and persistence of the plastic response, supporting θtACS + iTBS as a biologically grounded priming mechanism.

We propose that the observed enhancement arises because θtACS + iTBS modulates neuronal excitability in a manner that optimizes the temporal dynamics required for iTBS-induced plasticity. Because iTBS depends on spike-timing–dependent mechanisms involved in the formation of long-term potentiation (LTP) in the cerebellar cortex (*8*) and is highly state dependent (*19*), theta-frequency stimulation may refine the window for LTP induction within cerebellar microcircuits, particularly at mossy fiber-granule cell and parallel fiber-Purkinje cell synapses. In addition, theta pacing may refine spike timing within Golgi-granule-Purkinje loops, enhancing temporal filtering and promoting the selective propagation of coherent bursts (*20*–*23*). This coordinated state sharpens the precision of synaptic responses and broadens the population of neurons responsive to plasticity induction. Rather than acting additively, θtACS appears to prime cerebellar microcircuits for plasticity through the combined effects of temporal alignment and expanded neuronal recruitment.

Supporting this mechanism, the enhancement was both frequency specific and site specific. Cerebellar, but not M1, θtACS + iTBS increased corticospinal excitability and improved motor performance. In contrast, θtACS + iTBS over M1 had no measurable effect, whereas γtACS + iTBS enhanced excitability at M1 but not in the cerebellum, consistent with previous studies (*24*–*26*). These results highlight the importance of tailoring stimulation to the resonance characteristics of the targeted circuit.

A related question is whether the effects could reflect peripheral cranial nerve stimulation rather than cerebellar engagement (*27*). Our control experiment addressed this by applying θtACS over the trigeminal facial territory, matching somatosensory input but minimizing cerebellar current, whereas iTBS was delivered to the cerebellum. Trigeminal-site stimulation did not reproduce the facilitation observed with cerebellar θtACS + iTBS, indicating that the effects cannot be attributed to somatosensory or trigeminal pathways alone and supporting a cerebellar origin of plasticity enhancement.

In the stroke cohort, θtACS + iTBS produced substantially greater cerebellar-motor plasticity, indexed by a long-lasting increase in MEP amplitude, than γtACS + iTBS, whereas the θ-sham comparison did not reach significance. This pattern is expected as cerebellar iTBS alone produces relatively strong excitability increases in chronic stroke (*8*, *28*). From a clinical perspective, the behavioral results showed a clearer divergence: θtACS + iTBS yielded significantly greater 9HPT improvement than sham, with 10 of 14 individuals (71%) demonstrating faster performance. Improvements of ≈1 to 2 s are considered clinically meaningful indicators of enhanced dexterity in chronic stroke populations (*29*), and even modest improvements in task completion time may reflect meaningful gains in dexterity when observed after a single stimulation session in individuals with chronic deficits, where recovery often plateaus. Thus, even when physiological differentiation from sham is attenuated by an already-strong iTBS response, cerebellar theta-aligned stimulation yields measurable functional gains, supporting its translational potential.

In healthy participants, only cerebellar θtACS + iTBS produced a significant correlation between MEP enhancement and behavioral improvement, demonstrating that oscillation-aligned stimulation engages plasticity mechanisms with both physiological and functional relevance. The rapid effects underscore its translational promise as a noninvasive strategy for restoring dexterity and motor function.

These changes in corticospinal excitability likely arise through modulation of cerebello-thalamo-cortical pathways, particularly the dentate-thalamic projections to M1 (*30*). Although stimulation was applied to the cerebellum, the downstream physiological effects (captured via MEPs) emerged cortically, suggesting that theta entrainment may have enhanced LTP-related synaptic efficacy or excitability within these long-range circuits (*9*, *31*, *32*). A prior work has shown that the dentate nucleus projects to motor thalamic nuclei, which, in turn, influence pyramidal excitability in M1 (*33*). Our results are consistent with the idea that cerebellar theta stimulation creates a temporally receptive state for activity-dependent plasticity along this pathway.

These findings have important implications for stroke rehabilitation. Stroke remains a leading cause of long-term disability (*34*, *35*), often leaving individuals with persistent motor deficits. Current neuromodulation strategies typically target M1, which may be structurally damaged or functionally disconnected. The cerebellum, by contrast, is frequently spared and maintains extensive projections to frontal and parietal regions (*8*, *36*, *37*), making it a strategic target for neuromodulation. In healthy adults, θtACS + iTBS reduced γ-aminobutyric acid type A ($GABA_A$)–mediated intracortical inhibition, indicating effective engagement of interneuronal mechanisms that support plasticity induction. The reduction in SICI emerged at T15 rather than immediately after stimulation, suggesting a delayed adjustment of intracortical inhibitory circuits that may reflect homeostatic rebalancing of interneuron networks following increased corticospinal excitability. Patients with stroke did not show SICI modulation, consistent with extensive evidence that intracortical inhibitory mechanisms in the affected hemisphere are pathologically blunted (*38*, *39*). Thus, the absence of SICI change reflects known physiological constraints rather than a failure of the stimulation protocol, and critically, patients still demonstrated meaningful behavioral improvement.

The observed gains likely reflect both short-term adaptation and longer-term recalibration of cerebello-thalamo-cortical circuits that remain anatomically preserved after cortical strokes (*36*). This

pattern aligns with prior studies implicating cerebellar mechanisms in adaptive motor learning and sustained recalibration (*14*, *20*, *32*, *40*, *41*). Although our study focused on chronic stroke, rhythm-tuned neuromodulation may be even more effective in the subacute phase, when plasticity is heightened, and circuits are more responsive to intervention. By rhythmically engaging spared cerebellar networks, theta-aligned stimulation may help reinstate adaptive learning processes in impaired systems.

Future work should address individual variability in response. Factors such as lesion location, cerebellar structure, and network connectivity likely shape stimulation outcomes and the effectiveness of cerebello-thalamo-cortical engagement. Neuroimaging or electrophysiological biomarkers could help identify predictors of response and guide personalized stimulation, although this was not explored because imaging data were not available for all participants. Because iTBS-induced plasticity is state dependent (*19*), attentional factors may contribute to variability. Although arousal was not formally monitored, the frequency-specific and site-specific effects observed here argue against a non-specific attentional explanation as no modulation occurred during sham or γtACS sessions. Although these findings establish a link between cerebellar theta-aligned stimulation and motor plasticity, the study is limited by modest sample sizes and single-session interventions, which may underestimate cumulative or long-term effects. Interindividual variability in TMS-based corticospinal excitability is well documented in both healthy and clinical populations (*42*) and likely contributes to the spread of responses observed here. Although the 9HPT offers a sensitive index of dexterity, it captures only one dimension of motor function; future work should incorporate broader clinical measures and multisession protocols, including testing during the subacute phase when plasticity is heightened.

Although our findings are consistent with theta-frequency engagement of cerebellar circuits, we did not directly record cerebellar oscillatory activity. Noninvasive electrophysiological access to the cerebellum is limited by depth and geometry, making direct confirmation technically challenging. Our mechanistic interpretation is therefore inferential, grounded in the strong frequency and site specificity of the observed effects. Future work using cerebellar-optimized electroencephalography or magnetoencephalography, or advanced source-localization approaches, will be needed to directly characterize cerebellar theta dynamics.

More broadly, this work supports a principle of dynamic matching (*6*, *43*), in which tuning stimulation to a circuit's endogenous frequency enhances rather than overrides intrinsic dynamics. Cerebellar θtACS + iTBS exemplifies this approach, offering a biologically informed framework for targeted neuromodulation and a generalizable model for rhythm-aligned circuit repair in neurologic disease.

In conclusion, cerebellar θtACS + iTBS introduces a novel, resonance-guided strategy for enhancing motor plasticity in humans. This circuit-specific, frequency-matched, and scalable approach holds promise for restoring function in individuals with stroke or other movement disorders and underscores the translational potential of rhythm-informed neuromodulation.

MATERIALS AND METHODS

Study design

This study was designed to evaluate whether cerebellar theta-frequency stimulation enhances motor plasticity and behavior in both healthy individuals and patients with chronic stroke. We used a within-subject, randomized, sham-controlled, crossover design across six experiments. Phase 1 (experiments 1 to 4) included healthy adult participants. Phase 2 (experiments 5 and 6) involved patients with chronic stroke with mild upper limb motor impairments.

Sample sizes were initially determined a priori on the basis of published effect sizes from cerebellar neuromodulation and TMS plasticity studies in both healthy individuals and patients with stroke (*4*, *5*, *8*), which typically report large within-subject effects (Cohen's $d \approx 0.8$ to 1.0). Power estimates based on these values ($\alpha = 0.05$; power = 0.80) indicated that ~14 participants per experiment would provide sufficient sensitivity. Because effect-size estimates in the neuromodulation literature can vary substantially across protocols and populations, we elected to use equal or larger sample sizes across all experiments to obtain stable estimates of both mean effects and interindividual variability. All participants completed the full crossover design, and no interim analyses or stopping rules were applied. Data collection proceeded until all participants completed the full crossover protocol. No stopping rules were prespecified.

All participants were recruited from the IRCCS Santa Lucia Foundation Hospital and Ferrara University. All participants provided written informed consent in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of the IRCCS Santa Lucia Foundation Hospital (CE-FSL, Protocol CE/PROG.811, 19 February 2021) and the Area Vasta Emilia Centro (CE-AVEC, Protocol 117/2023/Oss/AOUFe, 15 February 2023). The study adhered to established TMS safety guidelines (*44*) and was recorded on the Open Science Framework (OSF; https://osf.io/wemn9; DOI: 10.17605/OSF.IO/WEMN9).

Eligibility criteria

Healthy participants were eligible if they were aged 18 to 40 years, with no history of neurological or psychiatric illness, no contraindications to noninvasive brain stimulation (e.g., history of seizures and metallic implants), not pregnant, and not currently using medications known to affect cortical excitability.

Stroke participants met the following inclusion criteria: age 18 to 80 years, single event of ischemic or hemorrhagic stroke (≥6 months before enrollment), unilateral lesion (left or right hemisphere), mild upper limb motor impairment, ability to complete the 9HPT with the paretic hand, and capacity to provide informed consent. Exclusion criteria included: severe spasticity (Modified Ashworth Scale > 2), apraxia or neglect interfering with motor testing, history of epilepsy or unprovoked seizures, implanted metallic or electronic devices (e.g., pacemaker and cochlear implant), pregnancy, magnetic resonance imaging (MRI)–confirmed cerebellar lesion, or current use of medications that affect cortical excitability (e.g., benzodiazepines and antiepileptics).

Endpoints and outcome measures

The primary physiological endpoint was MEP amplitude, measured using single-pulse TMS. Secondary endpoints included measures of intracortical inhibition and facilitation, specifically SICI [interstimulus interval (ISI) = 2 ms], ICF (ISI = 15 ms), and LICI (ISI = 100 ms), as well as behavioral performance on the 9HPT. All endpoints were prospectively defined. Holm correction was applied to control for multiple comparisons across time points and stimulation conditions. No formal outlier removal was performed, and all valid data points were retained in the analyses.

The study followed a within-subject, sham-controlled, crossover design across six experiments. Experiments 1, 2, and 5 involved three

stimulation conditions (sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS). Experiment 3 consisted of a single active condition (θtACS applied to the trigeminal afferent area) paired with cerebellar iTBS, serving as a somatosensory control for nonspecific facial stimulation. Experiments 4 and 6 included two conditions (θtACS + iTBS and sham-tACS + iTBS). Stimulation sessions were randomized, counterbalanced, and separated by a minimum of 72 hours to reduce carryover effects. All participants served as their own controls, allowing for repeated measures within individuals. Neurophysiological assessments included paired-pulse TMS protocols (SICI, ICF, and LICI) performed at baseline, immediately poststimulation (T0), and 15 min poststimulation (T15). In addition, single-pulse MEPs were recorded at 30 min poststimulation (T30) to assess the durability of plasticity effects. Behavioral assessments using the 9HPT were conducted before and 15 min after stimulation. For stroke participants, lesions were manually segmented from clinical MRI scans and normalized to Montreal Neurological Institute (MNI) space for group-level analysis.

The primary objective of the study was to determine whether cerebellar theta-frequency tACS paired with iTBS enhances motor plasticity and behavior relative to sham or frequency-mismatched stimulation. Secondary aims included evaluating the frequency and anatomical specificity of stimulation effects, examining correlations between physiological and behavioral changes, and assessing translational feasibility in chronic stroke. To minimize bias, participants were blinded to stimulation conditions, and experimenters responsible for neurophysiological and behavioral assessments were blinded during data collection and analysis. Stimulation delivery was automated and coded to ensure blinding integrity.

Phase 1: Healthy participants (experiments 1 to 4)

Phase 1 assessed the effects of cerebellar θtACS + iTBS on physiological and behavioral outcomes in healthy adults. Participants were recruited from the IRCCS Santa Lucia Foundation and the University of Ferrara. All experiments used a randomized, sham-controlled, double-blind, within-subject crossover design.

Experiment 1 investigated whether cerebellar theta-frequency stimulation enhances corticospinal excitability in a frequency-specific manner. Twenty healthy participants (12 female; mean age = 29.6 years; SD = 3.6) underwent three stimulation sessions, each involving iTBS applied to the right cerebellar hemisphere (contralateral to the dominant hand), paired with either sham, 5 Hz (θ), and 50 Hz (γ) tACS (Fig. 1, A to C). MEP amplitude was the primary outcome, with additional measurements of SICI, ICF, and LICI. Assessments were conducted at baseline, immediately poststimulation (T0), 15 min post (T15), and, for MEPs only, 30 min post (T30) (Fig. 1G).

Experiment 2 tested the anatomical specificity of stimulation by delivering the same three stimulation conditions (sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS) over the left primary motor cortex (M1) (Fig. 1E). Fourteen participants (10 female; mean age = 28.2 years; SD = 2.9) completed all sessions. Physiological outcomes mirrored those in experiment 1 and were assessed at identical time points (Fig. 1G).

Experiment 3 tested whether the observed effects could be explained by peripheral cranial nerve costimulation. A subset of ten participants from experiment 1 completed an additional trigeminal-site session, with its order counterbalanced relative to all other experimental sessions. In all sessions, iTBS was applied to the right cerebellar hemisphere whereas θtACS was delivered either to the cerebellar montage or to the maxillary branch of the trigeminal nerve (*45*) for the control condition (Fig. 1F). Physiological measurements were collected using the same procedures and time points as in experiments 1 and 2 (Fig. 1G).

Experiment 4 evaluated whether cerebellar θtACS + iTBS improves fine manual dexterity. Eighteen participants (10 female; mean age = 26.9 years; SD = 3.9) received θtACS + iTBS or sham-tACS + iTBS (Fig. 1, A and B) over the right cerebellar hemisphere combined with cerebellar iTBS in a randomized crossover design. Behavioral performance on the 9HPT, performed with the dominant hand, was measured before and 15 min after stimulation (Fig. 1H). MEPs were also recorded pre- and poststimulation to examine correlations between physiological and behavioral changes.

Allocations, demographic information, and stimulation parameters for the healthy participants engaged in phase 1 are provided in Table 1.

Phase 2: Stroke participants (experiments 5 and 6)

Phase 2 extended the findings to individuals with chronic stroke and upper limb motor impairment. Participants were recruited from the same sites and met predefined eligibility criteria. All stimulation was delivered to the cerebellar hemisphere contralateral to the lesioned cortex, consistent with the crossed cerebello-thalamo-cortical pathways through which the cerebellum modulates excitability in the opposite motor cortex. A randomized, double-blind, sham-controlled, within-subject crossover design was used.

Experiment 5 replicated the physiological protocol from experiment 1 in a clinical sample. Sixteen individuals with chronic stroke were enrolled, and twelve (3 female; mean age = 61.5 years; SD = 10.7) completed all three stimulation conditions (sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS). Single-pulse MEPs were recorded at baseline, T0, T15, and T30 (Fig. 1G) to assess the effects of θtACS + iTBS on corticospinal excitability through spared cerebello-thalamo-cortical pathways.

Experiment 6 assessed whether cerebellar theta stimulation improves motor performance in stroke. Fourteen participants (3 female; mean age = 60.7 years; SD = 9.0) completed both sham-tACS + iTBS and θtACS + iTBS sessions. The 9HPT was performed with the paretic hand before and 15 min after stimulation (Fig. 1H).

For all stroke participants, clinical MRI scans were used to segment lesions, which were then normalized to MNI space for group-level analysis. A lesion overlap map generated from 15 participants is presented in Fig. 5F. Lesions affected cortical regions such as the primary motor and premotor cortex and subcortical structures, including the internal capsule and basal ganglia; no participants had cerebellar damage.

Allocations, participant demographics and stimulation parameters for stroke subjects are provided in Table 2.

tACS + iTBS neuromodulation protocol

The combined tACS + iTBS protocol was adapted from prior works (*24*–*26*). tACS was delivered via saline-soaked sponge electrodes (5 cm by 5 cm) using a BrainStim stimulator (E.M.S., Bologna). θtACS (5 Hz) and γtACS (50 Hz) were applied at 2 mA. For cerebellar stimulation, the active electrode was positioned 3 cm lateral and 1 cm inferior to the inion. In healthy participants, stimulation was applied ipsilateral to the dominant hand, with the reference over the right

Table 1. Demographic and stimulation parameters for healthy participants. Participants across experiments 1 to 4 are listed with age, sex, handedness, motor threshold, and modeled electric field during iTBS. The AMT is reported as a percentage of the maximum stimulator output. Electric field (E-field) estimates are based on individual stimulation intensities.

Participant	Exp	Age	Sex	Dominant hand	AMT	iTBS E-field* (V/m)
I	1	37	M	Right	52	34
II	1	34	M	Right	45	29
III	1	31	F	Right	48	31
IV	1, 2	31	M	Left	69	45
V	1, 2	25	F	Right	50	34
VI	1, 2	28	F	Right	59	38
VII	1, 2	25	F	Right	40	28
VIII	1, 2, 3	34	M	Right	48	31
IX	1, 2	30	F	Right	41	27
X	1, 2	30	F	Right	49	33
XI	1, 2, 3	26	F	Right	41	26
XII	1, 2, 3	32	M	Right	64	42
XIII	1, 2, 3, 4	27	F	Right	63	40
XIV	1, 3	32	M	Right	41	27
XV	1, 3	30	M	Right	61	41
XVI	1, 3	26	F	Right	53	34
XVII	1, 3	25	F	Right	62	42
XVIII	1, 3, 4	34	F	Right	43	27
XIX	1, 3, 4	26	F	Right	53	34
XX	1, 4	29	M	Right	45	28
XXI	2	27	F	Right	55	36
XXII	2	26	M	Right	53	34
XXIII	2	29	F	Right	51	33
XXIV	2	25	F	Right	54	34
XXV	4	24	F	Right	69	45
XXVI	4	26	F	Right	43	27
XXVII	4	27	M	Right	48	31
XXVIII	4	34	M	Right	58	35
XXIX	4	26	F	Right	53	34
XXX	4	27	F	Right	60	37
XXXI	4	21	F	Right	51	33
XXXII	4	25	M	Right	65	42
XXXIII	4	23	M	Right	55	36
XXXIV	4	22	M	Right	50	34
XXXV	4	29	F	Right	58	35
XXXVI	4	34	F	Right	43	27
XXXVII	4	25	M	Right	55	36
XXXVIII	4	25	M	Right	71	44

*iTBS E-field was calculated using the individual TMS stimulation intensity via a computational model, and tACS was delivered with a current density of 0.08 mA/cm^2.

shoulder, to target the cerebellar hemisphere functionally connected to contralateral M1. In patients with stroke, the cerebellar hemisphere contralateral to the cortical lesion was targeted to engage the corresponding, anatomically spared cerebello-thalamo-cortical pathway. For M1 stimulation, the active electrode was positioned over the left M1 hotspot. For trigeminal tACS control stimulation, the active electrode was placed over the cheek (infraorbital region) to preferentially engage branches of the trigeminal nerve rather than cerebellar or cortical structures; the reference electrode was identical to the cerebellar montage. Sham stimulation involved a brief (5 s) ramp-up of θtACS.

iTBS was delivered using a Magstim Rapid2 with a figure-of-eight coil. The protocol consisted of 600 pulses delivered in 2-s trains (three pulses at 50 Hz repeated at 5 Hz), at 80% of the active motor threshold (AMT). Stimulation lasted 190 s and was synchronized with tACS

Table 2. Demographic and stimulation parameters for stroke participants. Stroke participants enrolled in experiments 5 and 6. Lesion sites were confirmed via MRI. The AMT is expressed as a percentage of the maximum stimulator output. Electric field (E-field) estimates were derived from individualized stimulation parameters using computational modeling.

Participant	Exp	Age	Sex	Dominant hand	Lesion site	Months from stroke onset	AMT	iTBS E-field* (V/m)
I	5	77	M	Right	Left pons	7	47	31
II	5	61	M	Right	Right parieto-temporal-insular	6	61	41
III	5	64	F	Right	Right centrum semiovale	25	60	38
IV	5	74	M	Right	Left internal capsule	90	49	31
V	5	52	M	Right	Left occipital	22	66	44
VI	5	46	M	Right	Right frontal-parietal	52	50	34
VII	5	52	F	Right	Right frontal-insular	16	44	28
VIII	5,6	74	M	Right	Left internal capsule	28	40	24
IX	5,6	56	M	Right	Medial right midbrain	6	58	38
X	5,6	71	M	Right	Right frontal-parietal-temporal	66	38	28
XI	5,6	49	F	Right	Right frontal + corona radiata	112	57	41
XII	5,6	62	M	Right	Right frontal-parietal-occipital	6	51	33
XIII	6	54	M	Left	Right insular + capsular-lenticular	50	61	41
XIV	6	52	M	Right	Right frontal-insular + deep capsular-lenticular-caudate	42	56	37
XV	6	72	M	Right	Left bulbar	237	55	36
XVI	6	61	M	Right	Left capsular-lenticular	35	65	42
XVII	6	54	F	Right	Right frontal-insular + deep capsular-lenticular-caudate	46	56	37
XVIII	6	55	F	Right	Left capsular-lenticular + corona radiata	168	68	45
XIX	6	51	M	Right	Left temporal-insular + corona radiata + centrum semiovale	23	49	33
XX	6	74	M	Right	Left lenticular + caudate nuclei	19	55	36
XXI	6	65	M	Right	Right capsular-thalamic	9	62	42

*iTBS E-field was calculated using the individual TMS stimulation intensity via a computational model, and tACS was delivered with a current density of 0.08 mA/cm^2.

using the BrainTrigger hardware and Signal software. The AMT was defined as the lowest intensity eliciting MEPs ≥ 200 μV during a voluntary contraction at 10% of the participant's maximal force, monitored via real-time visual feedback.

Neurophysiological assessment

Neurophysiological assessments were conducted using TMS in combination with surface electromyography (EMG). EMG signals were recorded from the right FDI muscle, amplified, and filtered using a Digitimer D360 amplifier (Digitimer Ltd., Welwyn Garden City, UK) with a band-pass filter ranging from 20 Hz to 2 kHz and digitized at a sampling rate of 5 kHz. Data were stored on a personal computer for offline analysis using the Signal software (version 6, Cambridge Electronic Design, UK).

Single-pulse TMS assessed corticospinal excitability; paired-pulse TMS assessed intracortical inhibition and facilitation using a Magstim 200^2 stimulator (BiStim^2 configuration) connected to a 70-mm figure-of-eight coil (Magstim Company Ltd., UK). The motor "hotspot" for the FDI muscle was defined as the scalp location over M1 that elicited the largest and most consistent MEPs. Stimulation intensity was individually calibrated and monitored throughout the experiment. The resting motor threshold (RMT) was defined as the minimum stimulus intensity required to evoke MEPs ≥ 50 μV in at least 5 of 10 trials in the relaxed FDI muscle.

Corticospinal excitability was assessed by recording 20 MEPs from the FDI hotspot in the left M1 (or from the affected M1 in stroke participants), using TMS intensity adjusted to produce responses of ~1 mV in amplitude (SI1mV). SICI and ICF were measured using

paired-pulse TMS with a conditioning stimulus (CS) set at 70% of RMT and a test stimulus (TS) adjusted to elicit ~1-mV MEPs at rest (SI1mV). SICI was assessed with an ISI of 2 ms; ICF was assessed with an ISI of 15 ms. LICI was evaluated using a suprathreshold CS at 120% of RMT and a TS set at SI1mV, with an ISI of 100 ms. For each protocol, 20 paired-pulse trials were recorded and averaged offline for analysis.

Behavioral assessment

Hand dexterity was evaluated via a sensorized 9HPT (*46*). Participants placed and removed nine pegs from a 3×3 matrix with their dominant hand. Patients with stroke used their paretic (affected) hand. Movement time was measured using a Hall effect sensor (DRV5053, Texas Instruments). Five trials were performed pre- and poststimulation, and the average completion time was used for analysis.

Statistical analysis

All statistical analyses were conducted using SPSS (IBM SPSS Statistics, version 24). To ensure baseline comparability across stimulation conditions, prestimulation MEP amplitudes were assessed using an RM-ANOVA for each experiment. Baseline corticospinal excitability was statistically equivalent across stimulation conditions in all experiments: Experiment 1: $F(2,38) = 2.47$, $P = 0.098$; experiment 2: $F(2,26) = 1.54$, $P = 0.232$; experiment 3: $F(1,9) = 2.38$, $P = 0.157$; experiment 4: $F(1,17) = 0.093$, $P = 0.764$; experiment 5: $F(2,22) = 0.173$, $P = 0.842$.

To assess stimulation effects, poststimulation MEP amplitudes were normalized to baseline (post/baseline ratio) and analyzed using RM-ANOVAs with the within-subject factors SESSION (sham-tACS + iTBS, θtACS + iTBS, and γtACS + iTBS) and TIME (T0, T15, and T30). This approach allowed evaluation of both frequency-specific and site-specific modulation of corticospinal excitability. Experiment 3 used a two-level RM-ANOVA with SESSION (cerebellar θtACS + iTBS versus trigeminal θtACS + iTBS) to determine whether physiological effects depended on cerebellar current flow rather than peripheral cranial nerve stimulation.

For intracortical inhibition and facilitation measures, separate RM-ANOVAs were conducted for each protocol: SICI, ICF, and LICI. This approach preserves neurophysiological specificity and aligns with prior works indicating distinct underlying mechanisms (*47*, *48*).

Behavioral performance on the 9HPT was analyzed using paired-samples *t* tests comparing movement-time improvements between θtACS + iTBS and sham-tACS + iTBS sessions. When normality assumptions were not met, Wilcoxon signed-rank tests were used as nonparametric complements. Brain-behavior associations were evaluated with Pearson's correlations between normalized MEP changes and behavioral improvement; these analyses were performed separately for theta and sham sessions.

To confirm anatomical and frequency specificity, supplementary linear mixed-effects models were conducted with fixed effects for SESSION and SITE (cerebellum versus M1) and random intercepts for SUBJECT. This analysis tested whether stimulation effects differed across target regions.

Statistical significance was set at $\alpha = 0.05$. Holm corrections were applied for post hoc comparisons to control for multiple testing. Data distributions were visually inspected and tested for normality using the Shapiro-Wilk test; all primary outcomes met criteria for approximate normality. No data were excluded as outliers, and no datasets contained missing values. Group-level outcomes are reported as mean ± SE unless otherwise specified.

To characterize interindividual variability and ensure robustness of the primary conclusions, supplementary analyses included responder classification, descriptive variability metrics (mean, SD, median, interquartile range, and coefficient of variation), and outlier screening at both subject and session levels. Nonparametric Wilcoxon signed-rank tests were additionally performed for behavioral outcomes and for physiological comparisons with non-normal residuals. These complementary analyses are reported exclusively in the Supplementary Materials and did not alter any primary statistical inference reported in the main text.

Supplementary Materials

This PDF file includes:

Supplementary Text
Tables S1 and S2
Fig. S1

REFERENCES

1. C. I. De Zeeuw, F. E. Hoebeek, M. Schonewille, Causes and consequences of oscillations in the cerebellar cortex. *Neuron* **58**, 655–658 (2008).
2. D. Popa, M. Spolidoro, R. D. Proville, N. Guyon, L. Belliveau, C. Léna, Functional role of the cerebellum in gamma-band synchronization of the sensory and motor cortices. *J. Neurosci.* **33**, 6552–6556 (2013).
3. D. Gandolfi, P. Lombardo, J. Mapelli, S. Solinas, E. D'Angelo, Theta-frequency resonance at the cerebellum input stage improves spike timing on the millisecond time-scale. *Front. Neural Circuits* **7**, 64 (2013).
4. D. Spampinato, E. Avci, J. Rothwell, L. Rocchi, Frequency-dependent modulation of cerebellar excitability during the application of non-invasive alternating current stimulation. *Brain Stimul.* **14**, 277–283 (2021).
5. A. Guerra, G. Paparella, M. Passaretti, D. Costa, D. Birreci, A. De Biase, D. Colella, L. Angelini, A. Cannavacciuolo, A. Berardelli, M. Bologna, Theta-tACS modulates cerebellar-related motor functions and cerebellar-cortical connectivity. *Clin. Neurophysiol.* **158**, 159–169 (2024).
6. G. Thut, P. Schyns, J. Gross, Entrainment of perceptually relevant brain oscillations by non-invasive rhythmic stimulation of the human brain. *Front. Psychol.* **2**, 170 (2011).
7. A. S. Asan, E. J. Lang, M. Sahin, Entrainment of cerebellar purkinje cells with directional AC electric fields in anesthetized rats. *Brain Stimul.* **13**, 1548–1558 (2020).
8. G. Koch, S. Bonnì, E. P. Casula, M. Iosa, S. Paolucci, M. C. Pellicciari, A. M. Cinnera, V. Ponzo, M. Maiella, S. Picazio, F. Sallustio, C. Caltagirone, Effect of cerebellar stimulation on gait and balance recovery in patients with hemiparetic stroke: A randomized clinical trial. *JAMA Neurol.* **76**, 170–178 (2019).
9. G. Koch, F. Mori, B. Marconi, C. Codecà, C. Pecchioli, S. Salerno, S. Torriero, E. Lo Gerfo, P. Mir, M. Oliveri, C. Caltagirone, Changes in intracortical circuits of the human motor cortex following theta burst stimulation of the lateral cerebellum. *Clin. Neurophysiol.* **119**, 2559–2569 (2008).
10. T. Popa, B. Velayudhan, C. Hubsch, S. Pradeep, E. Roze, M. Vidailhet, S. Meunier, A. Kishore, Cerebellar processing of sensory inputs primes motor cortex plasticity. *Cereb. Cortex* **23**, 305–314 (2013).
11. R. Courtemanche, Y. Lamarre, Local field potential oscillations in primate cerebellar cortex: Synchronization with cerebral cortex during active and passive expectancy. *J. Neurophysiol.* **93**, 2039–2052 (2005).
12. E. D'Angelo, T. Nieus, A. Maffei, S. Armano, P. Rossi, V. Taglietti, A. Fontana, G. Naldi, Theta-frequency bursting and resonance in cerebellar granule cells: Experimental evidence and modeling of a slow K^+-dependent mechanism. *J. Neurosci.* **21**, 759–770 (2001).
13. L. Mapelli, G. Gagliano, T. Soda, U. Laforenza, F. Moccia, E. U. D'Angelo, Granular layer neurons control cerebellar neurovascular coupling through an NMDA receptor/NO-dependent system. *J. Neurosci.* **37**, 1340–1351 (2017).
14. G. Koch, R. Esposito, C. Motta, E. P. Casula, F. Di Lorenzo, S. Bonnì, A. M. Cinnera, V. Ponzo, M. Maiella, S. Picazio, M. Assogna, F. Sallustio, C. Caltagirone, M. C. Pellicciari, Improving visuo-motor learning with cerebellar theta burst stimulation: Behavioral and neurophysiological evidence. *Neuroimage* **208**, 116424–116424 (2020).
15. M. J. Hartmann, J. M. Bower, Oscillatory activity in the cerebellar hemispheres of unrestrained rats. *J. Neurophysiol.* **80**, 1598–1604 (1998).
16. R. Courtemanche, J. C. Robinson, D. I. Aponte, Linking oscillations in cerebellar circuits. *Front. Neural Circuits* **7**, 125 (2013).

17. P. T. Huerta, J. E. Lisman, Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 in vitro. *Neuron* **15**, 1053–1063 (1995).
18. M. Hamada, N. Murase, A. Hasan, M. Balaratnam, J. C. Rothwell, The role of interneuron networks in driving human motor cortical plasticity. *Cereb. Cortex* **23**, 1593–1605 (2013).
19. A. Suppa, Y.-Z. Huang, K. Funke, M. C. Ridding, B. Cheeran, V. Di Lazzaro, U. Ziemann, J. C. Rothwell, Ten years of theta burst stimulation in humans: Established knowledge, unknowns and prospects. *Brain Stimul.* **9**, 323–335 (2016).
20. G. P. Dugué, N. Brunel, V. Hakim, E. Schwartz, M. Chat, M. Lévesque, R. Courtemanche, C. Léna, S. Dieudonné, Electrical coupling mediates tunable low-frequency oscillations and resonance in the cerebellar Golgi cell network. *Neuron* **61**, 126–139 (2009).
21. L. Ankri, Z. Husson, K. Pietrajtis, R. Proville, C. Léna, Y. Yarom, S. Dieudonné, M. Y. Uusisaari, A novel inhibitory nucleo-cortical circuit controls cerebellar Golgi cell activity. *Elife* **4**, e06262 (2015).
22. V. Lev-Ram, L. R. Makings, P. F. Keitz, J. P. Kao, R. Y. Tsien, Long-term depression in cerebellar Purkinje neurons results from coincidence of nitric oxide and depolarization-induced Ca^{2+} transients. *Neuron* **15**, 407–415 (1995).
23. E. D'Angelo, S. Solinas, J. Mapelli, D. Gandolfi, L. Mapelli, F. Prestori, The cerebellar Golgi cell and spatiotemporal organization of granular layer activity. *Front. Neural Circuits* **7**, 93 (2013).
24. A. Guerra, A. Suppa, M. Bologna, V. D'Onofrio, E. Bianchini, P. Brown, V. Di Lazzaro, A. Berardelli, Boosting the LTP-like plasticity effect of intermittent theta-burst stimulation using gamma transcranial alternating current stimulation. *Brain Stimul.* **11**, 734–742 (2018).
25. M. Maiella, E. P. Casula, I. Borghi, M. Assogna, A. D'Acunto, V. Pezzopane, L. Mencarelli, L. Rocchi, M. C. Pellicciari, G. Koch, Simultaneous transcranial electrical and magnetic stimulation boost gamma oscillations in the dorsolateral prefrontal cortex. *Sci. Rep.* **12**, 19391 (2022).
26. W.-Y. Liao, B. J. Hand, J. Cirillo, R. Sasaki, G. M. Opie, M. R. Goldsworthy, J. G. Semmler, Gamma transcranial alternating current stimulation has frequency-dependent effects on human motor cortex plasticity induced by theta-burst stimulation. *Eur. J. Neurosci.* **61**, e70018 (2025).
27. L.-Y. Liao, Y. Zhu, Q.-Y. Peng, Q. Gao, L. Liu, Q.-H. Wang, S.-H. Gao, Y. Tao, H. Huang, P.-D. Xu, C.-Y. Gao, Intermittent theta-burst stimulation for stroke: Primary motor cortex versus cerebellar stimulation: A randomized sham-controlled trial. *Stroke* **55**, 156–165 (2024).
28. H. M. Schambra, S. A. Hays, Vagus nerve stimulation for stroke rehabilitation: Neural substrates, neuromodulatory effects and therapeutic implications. *J. Physiol.* **603**, 723–735 (2025).
29. V. B. Raju, R. Hauer, M. Ghassemi, A. Banerjee, D. Kamper, B. D. Schmit, A. Vedantam, Virtual reality-based training to augment recovery of hand dexterity after surgery for degenerative cervical myelopathy. *Sci. Rep.* **15**, 22785 (2025).
30. F. A. Middleton, P. L. Strick, Basal ganglia and cerebellar loops: Motor and cognitive circuits. *Brain Res. Rev.* **31**, 236–250 (2000).
31. R. L. Buckner, F. M. Krienen, A. Castellanos, J. C. Diaz, B. T. T. Yeo, The organization of the human cerebellum estimated by intrinsic functional connectivity. *J. Neurophysiol.* **106**, 2322–2345 (2011).
32. D. Spampinato, P. Celnik, Deconstructing skill learning and its physiological mechanisms. *Cortex* **104**, 90–102 (2018).
33. Z. J. Daskalakis, G. O. Paradiso, B. K. Christensen, P. B. Fitzgerald, C. Gunraj, R. Chen, Exploring the connectivity between the cerebellum and motor cortex in humans. *J. Physiol.* **557**, 689–700 (2004).
34. J. W. Krakauer, "The applicability of motor learning to neurorehabilitation" in *Oxford Textbook of Neurorehabilitation*, V. Dietz, N. Ward, Eds. (Oxford Univ. Press, 2015).
35. J. Veldema, A. Gharabaghi, Non-invasive brain stimulation for improving gait, balance, and lower limbs motor function in stroke. *J. Neuroeng. Rehabil.* **19**, 84 (2022).
36. R. M. Kelly, P. L. Strick, Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *J. Neurosci.* **23**, 8432–8444 (2003).
37. A. R. Luft, R. F. Macko, L. W. Forrester, F. Villagra, F. Ivey, J. D. Sorkin, J. Whitall, S. McCombe-Waller, L. Katzel, A. P. Goldberg, D. F. Hanley, Treadmill exercise activates subcortical neural networks and improves walking after stroke: A randomized controlled trial. *Stroke* **39**, 3341–3350 (2008).
38. F. C. Hummel, B. Steven, J. Hoppe, K. Heise, G. Thomalla, L. G. Cohen, C. Gerloff, Deficient intracortical inhibition (SICI) during movement preparation after chronic stroke. *Neurology* **72**, 1766–1772 (2009).
39. M. N. McDonnell, C. M. Stinear, TMS measures of motor cortex function after stroke: A meta-analysis. *Brain Stimul.* **10**, 721–734 (2017).
40. J. Izawa, S. E. Criscimagna-Hemminger, R. Shadmehr, Cerebellar contributions to reach adaptation and learning sensory consequences of action. *J. Neurosci.* **32**, 4230–4239 (2012).
41. M. Coesmans, J. T. Weber, C. I. De Zeeuw, C. Hansel, Bidirectional parallel fiber plasticity in the cerebellum under climbing fiber control. *Neuron* **44**, 691–700 (2004).
42. C. Bigoni, S. Pagnamenta, A. Cadic-Melchior, M. Bevilacqua, S. Harquel, E. Raffin, F. C. Hummel, MEP and TEP features variability: Is it just the brain-state? *J. Neural Eng.* **21**, 016011 (2024).
43. P. Fries, Rhythms for cognition: Communication through coherence. *Neuron* **88**, 220–235 (2015).
44. S. Rossi, A. Antal, S. Bestmann, M. Bikson, C. Brewer, J. Brockmöller, L. L. Carpenter, M. Cincotta, R. Chen, J. D. Daskalakis, V. Di Lazzaro, M. D. Fox, M. S. George, D. Gilbert, V. K. Kimiskidis, G. Koch, R. J. Ilmoniemi, J. P. Lefaucheur, L. Leocani, S. H. Lisanby, C. Miniussi, F. Padberg, A. Pascual-Leone, W. Paulus, A. V. Peterchev, A. Quartarone, A. Rotenberg, J. Rothwell, P. M. Rossini, E. Santarnecchi, M. M. Shafi, H. R. Siebner, Y. Ugawa, E. M. Wassermann, A. Zangen, U. Ziemann, M. Hallett, Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. *Clin. Neurophysiol.* **132**, 269–306 (2021).
45. S. Vanneste, A. Mohan, H. B. Yoo, Y. Huang, A. M. Luckey, S. L. McLeod, M. N. Tabet, R. R. Souza, C. K. McIntyre, S. Chapman, I. H. Robertson, W. T. To, The peripheral effect of direct current stimulation on brain circuits involving memory. *Sci. Adv.* **6**, eaax9538 (2020).
46. V. Mathiowetz, G. Volland, N. Kashman, K. Weber, Adult norms for the box and block test of manual dexterity. *Am. J. Occup. Ther.* **39**, 386–391 (1985).
47. T. Kujirai, M. D. Caramia, J. C. Rothwell, B. L. Day, P. D. Thompson, A. Ferbert, S. Wroe, P. Asselman, C. D. Marsden, Corticocortical inhibition in human motor cortex. *J. Physiol.* **471**, 501–519 (1993).
48. P.-Y. Fong, D. Spampinato, L. Rocchi, R. Hannah, Y. Teng, A. Di Santo, M. Shoura, K. Bhatia, J. C. Rothwell, Two forms of short-interval intracortical inhibition in human motor cortex. *Brain Stimul.* **14**, 1340–1352 (2021).

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Elevating cerebellar theta oscillations boosts noninvasively induced motor plasticity

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