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A Comparison of Primed Low-Frequency Repetitive Transcranial Magnetic Stimulation Treatments In Chronic Stroke

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Abstract

Background—Preceding low-frequency repetitive transcranial magnetic stimulation (rTMS) with a bout of high-frequency rTMS called priming potentiates the after-effects of the former in healthy adults. The utility of primed rTMS in stroke remains under-explored despite its theoretical benefits in enhancing cortical excitability and motor function.

Objective—To ascertain the efficacy of priming in chronic stroke by comparing changes in cortical excitability and paretic hand function following three types of primed low-frequency rTMS treatments.

Methods—Eleven individuals with chronic stroke participated in this repeated-measures study receiving three treatments to the contralesional primary motor cortex in randomized order: 6 Hz primed 1 Hz rTMS, 1 Hz primed 1 Hz rTMS, and sham 6 Hz primed active 1 Hz rTMS. Within- and between-treatment differences from baseline in cortical excitability and paretic hand function from baseline were analyzed using mixed effects linear models.

Results—6 Hz primed 1 Hz rTMS produced significant within-treatment differences from baseline in ipsilesional cortical silent period (CSP) duration and short-interval intracortical inhibition. Compared to 1 Hz priming and sham 6 Hz priming of 1 Hz rTMS, active 6 Hz priming generated significantly greater decreases in **ipsilesional** CSP duration. These heightened effects were not observed for intracortical facilitation or interhemispheric inhibition excitability measures.

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Conclusion—Our findings demonstrate the efficacy of 6 Hz primed 1 Hz rTMS in probing homeostatic plasticity mechanisms in the stroke brain as best demonstrated by differences CSP duration and SICI from baseline. Though 6 Hz priming did not universally enhance cortical excitability across measures, our findings pose important implications in non-invasive brain stimulation application in stroke rehabilitation.

Keywords

stroke; priming; repetitive transcranial magnetic stimulation; metaplasticity; homeostatic plasticity

Introduction

Several challenges underlie stroke recovery. In addition to the death and destruction of neural substrate, the neural environment is radically different. Neurotransmitter fluctuations [1] and modulations in cortical excitability [2,3], including imbalances in interhemispheric inhibition [4], impede recovery. Developing innovative therapeutic targets and treatments is dependent on the elucidation of this intricate and dynamic post-stroke neural environment.

Application of non-invasive brain stimulation (NIBS) in stroke has advanced our understanding of neural reorganization. Various forms of NIBS like repetitive transcranial magnetic stimulation (rTMS), theta-burst stimulation (TBS), and transcranial direct current stimulation (TDCS) also show promise as potential adjuncts to traditional therapy. The predominant aim of NIBS in stroke is enhanced excitability of the ipsilesional primary motor cortex (M1) achieved with either facilitatory stimulation (i.e. high-frequency rTMS, intermittent TBS, and anodal TDCS) to the ipsilesional M1 or suppressive stimulation (i.e. low-frequency rTMS, continuous TBS, and cathodal TDCS) to the contralesional M1. Both approaches have resulted in improvements in paretic hand function [2,5–10]. Recent investigation has shown that preceding conditioning NIBS with an extra bout of stimulation, referred to as *priming*, potentiates the after-effects of the former particularly when the directionality (i.e. facilitatory or suppressive effect) of the priming stimulation is opposite to that of the conditioning stimulation [11–13].

The utility of priming is based on the Bienenstock-Cooper-Munro (BCM) theory of bidirectional synaptic plasticity that introduced a sliding synaptic threshold model [14].

Recent postsynaptic firing drives cellular and molecular processes that elevate the threshold for future potentiation while lowering the threshold for future depression. The reverse is true for recent postsynaptic depression. This negative feedback loop serves as the framework for homeostatic metaplasticity, an advanced type of plasticity that regulates long-term potentiation and depression [15,16]. Such ‘synaptic wisdom,’ operating via N-methyl-D-aspartate receptor modification [17] upholds network specificity from previous learning while maintaining flexibility for future experience-dependent learning.

Animal studies have confirmed the BCM rule [18–20]. Translation of these homeostatic principles to humans by using different combinations of NIBS [12,13,21–23] or by pairing NIBS with subsequent motor learning [24] has also demonstrated priming-induced shifts in Hebbian plasticity. In particular, Iyer et al. observed an enhancement of 1 Hz rTMS

suppression with the addition of 6 Hz rTMS priming in comparison to sham 6 Hz priming [11].

Potentiating the effects of 1 Hz rTMS with priming may prove beneficial in rTMS application in stroke. We previously verified the safety and feasibility of 6 Hz primed 1 Hz rTMS to contralesional M1 [25,26], as well as stroke characteristics distinguishing responders from nonresponders [27]. Yet, the efficacy of priming to probe homeostatic-like mechanisms of plasticity in stroke remains under-explored. The objective of this study was to compare changes in cortical excitability and paretic hand function following three types of primed 1 Hz rTMS in adults with stroke. We hypothesized that active 6 Hz priming, compared to active 1 Hz and sham 6 Hz priming, would result in significantly greater differences from baseline in cortical excitability, consistent with disinhibition of the ipsilesional hemisphere, and in paretic hand function.

Materials and Methods

Participants

We recruited individuals at least 18 years of age with ischemic or hemorrhagic stroke (duration ≥ 6 months) involving cortical and/or subcortical regions. Additional inclusion criteria included a Mini-mental State Examination score ≥ 24 out of 30 [28], presence of a resting motor-evoked potential from ipsilesional and contralesional hemispheres, and at least 10 degrees of active extension at the paretic metacarpophalangeal joint. Exclusionary criteria included seizure occurrence within the past two years, pregnancy, indwelling metal, implanted medical devices, and usage of tricyclic anti-depressants or neuroleptics. A neurologist reviewed pertinent medical and imaging records. Participants completed an in-person screen to assess TMS response, motor impairment (Upper Extremity Fugl-Meyer) [29] neurological deficit (National Institutes of Health Stroke Scale)[30], handedness (Edinburgh Handedness Inventory)[31] and mood (Beck Depression Inventory-II)[32]. The in-person screen coincided with the first day of study participation. This study was approved by the US Food and Drug Administration and the University of Minnesota Institutional Review Board and Clinical and Translational Science Institute. All participants provided written informed consent.

Study Design

This study utilized a repeated-measures crossover design to compare three different rTMS treatments (Figure 1). Over five weeks, participants received one session of each of the following treatments in randomized order: A. active 6 Hz priming + active 1 Hz rTMS, B. active 1 Hz priming + active 1 Hz rTMS, and C. sham 6 Hz priming + active 1 Hz rTMS. Participants and tester were blinded to treatment order.

Testing Procedures

Cortical Excitability—The primary outcome measure of this study was difference in cortical excitability from baseline (average of pretest 1 and 2) at posttests 1, 2, and 3. We measured interhemispheric inhibition (IHI), short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and cortical silent period (CSP) using two Magstim 200²

stimulators with a Bistim connecting module, a Bistim Trigger Box and two 50 mm figure-eight coils (IHI testing), and one 70 mm figure-eight coil (Magstim Company Ltd, Spring Gardens, UK). SICI, ICF, and CSP measures were taken from the ipsilesional hemisphere while IHI testing involved bilateral hemisphere measurements.

Participants donned earplugs, and the tester applied surface electrodes to the paretic and non-paretic first dorsal interossei (FDI). The reference electrodes were located on the dorsum of the paretic and non-paretic hands. Electromyography (EMG) signals were amplified and collected with a Cadwell Sierra Wave EMG device (Cadwell Laboratories, Kennewick, WA) equipped with a 20 Hz to 2.0 kHz bandpass filter and 60 Hz notch filter. The sampling rate was 6.4 kHz. EMG signals were recorded for 300 ms for each trial with a 30 ms pre-trigger duration. Investigators monitored background EMG activity throughout the duration of threshold and excitability testing to ensure that all measures, with the exception of CSP testing, were done at rest. Trace recordings are provided in Figure 2. EMG recordings were stored on a laptop computer for offline analysis.

Threshold Determination—The participant sat in a reclining chair while the tester made temporary markings on the participant's scalp to denote their hotspot, defined as the optimal location on the scalp that elicited a MEP from the FDI with the lowest stimulation intensity. The tester positioned the coil handle 45 degrees posterolaterally to the mid-sagittal line on the cranium on the motor hotspot region and delivered single TMS pulses to determine the participant's resting motor threshold (RMT) and 0.50 mV threshold. The RMT was the lowest stimulus intensity required to produce a 50 μ V MEP in 3 of 5 trials [33]. The 0.50 mV threshold was the lowest stimulus intensity required to produce a 0.50 mV MEP in 3 of 5 trials. Maximum intensity was 100% of machine output. The tester determined the RMT and 0.50 mV threshold for both hemispheres using two 50 mm figure-of-eight coils (one for each hemisphere) during IHI testing, and used the 70 mm coil to determine the RMT and 0.50 mV threshold in the ipsilesional hemisphere for SICI, ICF, and CSP testing.

Interhemispheric Inhibition—Similar to the IHI testing protocol described by Kirton et al. [34], the tester delivered a suprathreshold (0.50 mV threshold) conditioning pulse to the motor hotspot of one hemisphere followed 10 ms later by a suprathreshold test pulse to the motor hotspot on the opposite hemisphere. If a 0.50 mV threshold could not be obtained, the pulses were 120% of the participant's RMT. In randomized order, the tester delivered 10 trials of each of the following blocks: 1) single test pulse to contralesional M1, 2) single test pulse to ipsilesional M1, 3) conditioning pulse to ipsilesional M1 and test pulse to contralesional M1, and 4) conditioning pulse to contralesional M1 and test pulse to ipsilesional M1. The 40 trials were separated by approximately 6 seconds. We calculated the ratio of mean amplitude of paired-pulse MEPs to the mean amplitude of single-pulse MEPs for both hemispheres.

Short-Interval Intracortical Inhibition & Intracortical Inhibition—The tester applied a subthreshold (80% RMT) conditioning pulse and a suprathreshold (0.50 mV threshold or 120% RMT) test pulse to the participant's ipsilesional motor hotspot. Pulses were separated by either 3 ms to assess GABA_A-mediated SICI [35] or by 15 ms to measure ICF which represents glutamatergic synaptic activity [36]. Participants received 10 single-

pulse trials, 10 paired-pulse trials with an ISI of 3 ms, and 10 paired-pulse trials with an ISI of 15 ms in randomized order. Paired-pulse to single-pulse MEP amplitude ratios were constructed offline.

Cortical Silent Period—The participant sat upright with their paretic index finger positioned in a ring attached to a S-beam load cell (Interface Inc., Scottsdale AZ) and completed three trials of maximal isometric abduction using their paretic FDI. The voltage signal from the load cell was measured using WinDaq software (WinDaq, Akron, OH) and the highest force output produced during the three trials was determined. The participant performed a sub-max (30%) isometric contraction as the tester delivered 10 single suprathereshold (150% RMT) pulses to their ipsilesional motor hotspot. Pulses were separated by 20 seconds. The CSP duration was calculated offline as the time from the stimulus artifact to the resurgence of EMG activity at least 50% of the average prestimulus EMG activity.

Paretic Hand Function—Our secondary outcome measure to assess potential behavioral change was the Box and Block test. The test requires individuals to retrieve 2.5 cm³ blocks and transfer the blocks one at a time from one compartment to another. Participants completed three 60-second trials. The number of blocks successfully transferred was counted and an average was computed. The Box and Block shows high test-retest reliability (intraclass correlation coefficient = 0.96–0.98) in stroke [37]. Minimal detectable change for the paretic and non-paretic hands is 5.5 and 7.8 blocks per minute, respectively [37].

Safety—The tester/treater collected blood pressure and pulse readings from participants at the beginning of each visit. Weight was measured at the start of each week excluding washouts. Participants completed the Digit Span test at each visit as an assessment of short-term memory. The investigator recited a string of numbers. Participants recited the numerical sequences until they missed two consecutive sequences of the same length. To ensure no adverse physical detriments of the non-paretic hand, participants repeated the Box and Block Test using their non-paretic hand. At the start and end of each visit, participants provided a report of symptoms. A brief physician exam occurred on the first and last day of study participation.

Treatment Procedures

The treater determined the participant's contralesional FDI motor hotspot and RMT using a Magstim Rapid² stimulator and 70 mm air film coil (Magstim Company Limited, Dyfed, UK). Participants wore earplugs and surface electrodes on their non-paretic FDI, biceps brachii, extensor digitorum, and gastrocnemius muscles to monitor for abnormal muscle activity indicative of possible seizure onset. Following threshold determination, the investigator either replaced the active coil with the sham coil in preparation for sham 6 Hz priming or simulated changing the coil for the active 6 Hz and 1 Hz priming. At the conclusion of the priming, the investigator then either replaced the sham coil with the active coil or simulated changing the coil in preparation for the active 1 Hz conditioning.

All treatments included a 10-minute session of active 1 Hz rTMS (600 pulses, 90% RMT). The preceding priming session entailed either 10 minutes of active intermittent 6 Hz rTMS (5-second train, 2 trains/minute, 25-second intertrain interval; 600 total pulses, 90% RMT), 10 minutes of active 1 Hz rTMS (600 pulses, 90% RMT), or 10 minutes of sham intermittent 6 Hz rTMS (5-second train, 2 trains/minute, 25-second intertrain interval; 600 pulses). All treatments were applied to the participant's contralesional motor hotspot. We used a 70 mm sham air film coil for sham priming. The coil produced similar auditory and tactile sensations as the active coil but did not generate a magnetic field.

Statistical Analysis

All statistical procedures were done with SAS® 9.3 (SAS Institute Inc., Cary, NC). The study was a three-period (week 1, 3, 5), three-treatment (A, B, C) repeated-measures crossover design. Participants were randomly assigned to 1 of 6 treatment sequences (i.e. ABC, ACB, BAC, BCA, CAB, CBA). To examine differences in cortical excitability and paretic hand function from baseline (i.e. within-treatment change) as well as to compare differences from baseline between treatments (i.e. between-treatment change), we used a mixed-effects linear model. The fixed effects were treatment, period, and sequence. A random intercept is included for each participant to model within-subject correlation (Appendix A). Specifically, we used SAS PROC MIXED with a compound symmetry covariance structure. The EMPIRICAL option was used to yield robust standard errors for parameters in the presence of model misspecification.

Prior to assessing treatment effects, we checked if baseline measurements demonstrated significant treatment carryover and period effects. We also screened for unequal treatment carryover and treatment-by-period interactions by running the model with treatment, period, and treatment sequence as fixed effects. The F-test for treatment sequence was used to test for carryover and treatment-by-period interaction. Due to the pilot-nature of this study, we did not adjust *p*-values to account for multiple *t*-test comparisons [38,39]. A *p*-value 0.05 was considered statistically significant.

Results

Subjects

Eleven individuals participated in the study (3 females, mean $\pm$ standard deviation (SD) age = 66 ± 9.4 years, range: 48–84 years, mean time post-stroke = 57.4 ± 52.0 months, range: 12–159 months). Tables 1 and 2 provide additional participant demographics and screening results. Ten participants completed the study. One participant dropped out **after completing one full week** due to medical issues unrelated to the study. The data from this participant was retained and included in the analyses. All participants remained blinded to their treatment order and tolerated the rTMS interventions. There were no decrements in non-paretic hand function and short-term memory.

Cortical Excitability

Treatment A refers to 6 Hz primed 1 Hz rTMS, treatment B refers to 1 Hz primed 1 Hz rTMS, and treatment C refers to sham 6 Hz primed active 1 Hz rTMS. With the exception of

CSP duration, all cortical excitability measures in this study were based on the ratio of MEP amplitudes from paired-pulse to a single-pulse TMS. Amplitude units (millivolts) therefore cancel. Paired-pulse to single-pulse MEP amplitude ratios greater than 1.0 indicate facilitation; whereas, ratios below 1.0 indicate suppression. The findings reported below are the posttest – baseline differences in paired-pulse to single-pulse MEP ratio, CSP duration, and Box and Block score. Group baseline averages for cortical excitability and behavioral measures are provided in Table 3. A complete listing of model effects for screening procedures (i.e. sequence/carryover and period effects and treatment-by-period interactions) and treatment effects are reported in Supplementary Tables 1 and 2.

Contra-to-Ipsilesional IHI—A significant decrease in contra-to-ipsilesional IHI occurred at posttest 1 for treatment C (Least Squares Mean (LSM) $\pm$ Standard Error (SE) = $.290 \pm 0.123$, $t = 2.350$, $p = 0.032$, Figure 3a). However, analysis of treatment effects revealed significant carryover at posttest 1 ($F_{(2,14)} = 18.57$, $p = 0.0001$), meaning that treatment effects from the previous week persisted beyond the one-week washout period. After accounting for the significant carryover effect, the treatment effect was no longer significant ($F_{(2,16)} = 1.86$, $p = 0.281$). No significant treatment effects occurred at posttest 2 ($F_{(2,15)} = 1.39$, $p = 0.278$) nor at posttest 3 ($F_{(2,15)} = 0.67$, $p = 0.528$).

Ipsi-to-Contralesional Interhemispheric Inhibition—No significant within-treatment differences from baseline occurred at posttest 1 ($F_{(2,16)} = 0.50$, $p = 0.614$), posttest 2 ($F_{(2,15)} = 2.45$, $p = 0.120$), and posttest 3 ($F_{(2,15)} = 2.71$, $p = 0.099$). There was a trend of a greater increase in ipsi-to-contralesional IHI from baseline following treatment A compared to treatment C on posttest 2 ($A - C = -0.154 \pm 0.075$, $t = -2.050$, $p = 0.058$, Figure 3b). Treatment C yielded a significantly greater difference from baseline in IHI compared to treatment A on posttest 3 ($A - C = 0.208 \pm 0.090$, $t = 2.320$, $p = 0.035$).

Short-Interval Intracortical Inhibition—A significant treatment effect, indicating a decrease in SICI from baseline (i.e. a positive posttest – baseline difference in PP/SP ratio), was present at posttest 1 ($F_{(2,15)} = 3.76$, $p = 0.047$) for Treatment A (0.151 ± 0.062 , $t = 2.450$, $p = 0.027$, Figure 4a). There was a trend towards treatment A producing greater decreases in SICI compared to treatments B ($A - B = 0.209 \pm 0.099$, $t = 2.100$, $p = 0.053$) and C ($A - C = 0.144 \pm 0.073$, $t = 1.970$, $p = 0.068$) at posttest 1.

Intracortical Facilitation—No significant differences from baseline were found for ICF (Figure 4b) at posttest 1 ($F_{(2,16)} = 0.42$, $p = 0.666$), posttest 2 ($F_{(2,16)} = 1.24$, $p = 0.315$), and posttest 3 ($F_{(2,16)} = 0.15$, $p = 0.866$). A significant period effect for baselines ($F_{(2,48)} = 5.61$, $p = 0.006$) occurred at week 5 (0.476 ± 0.222 , $t = 2.150$, $p = 0.037$) in addition to significant carryover at posttest 2 ($F_{(2,14)} = 4.48$, $p = 0.031$).

Cortical Silent Period—A significant within-treatment effect from baseline ($F_{(2,14)} = 6.22$, $p = 0.012$) for treatment A occurred on posttest 3 (-32.809 ± 13.569 ms, $t = -2.420$, $p = 0.030$, Figure 5). Treatment A produced significantly greater difference in CSP duration from baseline compared to treatment B on posttest 2 ($A - B = -31.590 \pm 13.565$ ms, $t = -2.330$, $p = 0.035$) and on posttest 3 ($A - B = -46.181 \pm 17.516$ ms, $t = -2.640$, $p = 0.020$).

A significant between-treatment difference was also found between treatments A and C on posttest 3 ($A - C = -35.967 \pm 12.492$ ms, $t = -2.880$, $p = 0.012$).

Box and Block—Participants demonstrated a significant improvement in Box and Block performance from baseline following treatment A at posttests 2 (1.83 ± 0.849 blocks, $t = 2.150$, $p = 0.047$, Figure 6) and 3 (2.47 ± 0.908 blocks, $t = 2.730$, $p = 0.015$) and following treatment B at posttest 3 (3.16 ± 1.10 blocks, $t = 2.860$, $p = 0.011$). However, assessment of baselines indicated significant treatment carryover ($F_{(3,46)} = 3.27$, $p = 0.029$) following treatment A (5.52 ± 2.19 blocks, $t = 2.520$, $p = 0.047$) and a significant period main effect ($F_{(2,48)} = 9.76$, $p = 0.0003$) at weeks 3 (3.70 ± 1.28 , $t = 2.880$, $p = 0.006$) and 5 (5.03 ± 1.21 , $t = 4.160$, $p < 0.001$). Analysis of treatment effects showed a significant carryover effect for posttest 1 ($F_{(2,14)} = 8.53$, $p = 0.004$) and a significant treatment-by-period interaction for posttest 3 ($F_{(2,14)} = 4.45$, $p = 0.020$).

Discussion

This study investigated the utility of different forms of priming rTMS prior to active 1 Hz conditioning rTMS delivery in stroke. After screening baselines and treatment effects, we found that 6 Hz priming produced significant decreases in ipsilesional CSP duration and ipsilesional SICI from baseline. Further, 6 Hz priming generated significantly greater decreases in CSP duration than 1 Hz and sham 6 Hz priming. Sham 6 Hz priming resulted in significantly greater increases in ipsi-to-contralesional IHI than 6 Hz priming at posttest 3.

The multitude of priming literature in stroke focuses on the use of NIBS to prime subsequent behavioral training [40–44]. Kakuda et al. [44] confirmed the safety and feasibility of 6 Hz primed 1 Hz rTMS with subsequent occupational therapy. The priming event was the combined 6 Hz + 1 Hz rTMS and the ensuing conditioning event was occupational therapy. Two other studies in stroke utilized NIBS to prime *and* condition the brain [45,46]. In these investigations, participants received 1 Hz rTMS priming to contralateral M1 and subsequent intermittent TBS conditioning to ipsilesional M1. Researchers found significant changes in contra- and ipsilesional motor map areas [45] and paretic hand function [45,46] compared to sham control conditions and in individuals receiving ipsilesional intermittent TBS followed by contralateral 1 Hz rTMS [46]. The distinctive features of our study were the examination of interhemispheric and intracortical circuitry with priming/conditioning given exclusively to contralateral M1.

Significant modulations in GABAergic inhibitory circuits, depicted by reduced CSP duration and SICI, following 6 Hz priming is consistent with prior work by Siebner et al. that observed priming-induced shifts in intracortical inhibition following TDCS primed 1 Hz rTMS [23]. Modulations in intracortical inhibition from 6 Hz priming support the existence of homeostatic-like metaplasticity in the stroke brain. Consistent with the work of Ragert et al. that demonstrated homeostatic mechanisms functioning between hemispheres following priming/conditioning stimulation to opposite M1s [47], our results also reflect the expansive post-synaptic landscape of metaplasticity. Though synapse-specific examination is beyond the scope of this study, we gather that synapses directly involved during priming (i.e.

homosynaptic metaplasticity) along with those that were not (i.e. heterosynaptic metaplasticity) shaped the priming/conditioning stimulation interaction.

Yet, we cannot disregard possible contributions from calcium-mediated gating and/or anti-gating mechanisms [48,49]. Nitsche et al. observed only homeostatic interactions between TDCS primed PAS when the two were delivered simultaneously [50]. When TDCS and PAS were delivered consecutively, Nitsche and colleagues observed a synergistic effect consistent with underlying gating mechanisms. Since the majority of our cortical excitability measures probed ipsilesional excitability following treatment to contralesional M1, it is possible that the changes in CSP duration and SICI might have resulted indirectly from changes in contra- and/or ipsilesional GABAergic inhibition immediately following 6 Hz priming [50]. Expanding our excitability measures to contralesional M1 and studying the effects of priming alone would help elucidate gating contributions. Discerning the exact mechanism of homeostatic plasticity regulation is challenging as metaplasticity and gating likely function simultaneously.

Consistent with our hypothesis, 6 Hz priming led to greater differences in cortical excitability from baseline compared to 1 Hz and sham 6 Hz priming. In fact, the relationship between 6 Hz and 1 Hz CSP differences from baseline appear anti-correlated with 1 Hz primed 1 Hz rTMS depicting a homeostatic interaction in the opposite direction from 6 Hz primed 1 Hz rTMS. In the case of contra-to-ipsilesional IHI, 1 Hz rTMS depicted similar decreases, albeit lesser, than 6 Hz priming thus demonstrating a non-homeostatic interaction with 1 Hz rTMS conditioning.

Earlier work has shown ipsilesional M1 disinhibition after low-frequency rTMS to contralesional M1 [2,5,51]. On average, participants demonstrated a 2–3% decrease in ipsilesional M1 RMT from baseline following both active 6 Hz and sham 6 Hz primed 1 Hz rTMS (data not shown). We hypothesized that sham 6 Hz primed 1 Hz rTMS would result in similar, albeit less, disinhibition to ipsilesional M1 than active 6 Hz primed 1 Hz rTMS. In one case, however, the magnitude of change from baseline, as exemplified at posttest 3 for ipsi-to-contralesional IHI, was greater for sham vs. active 6 Hz priming. Based on the above findings, it is likely that sham priming 1 Hz rTMS inhibited contralesional M1 *and* disinhibited ipsilesional M1 to a greater degree than 6 Hz primed 1 Hz rTMS at posttest 3.

Inter-individual variability in responsiveness to NIBS [52–55] *and* in homeostatic responsiveness to priming stimulation may partially explain the discrepancies in 6 Hz priming efficacy. The val⁶⁶met polymorphism on the brain-derived neurotrophic factor (BDNF) gene may impact homeostatic responsiveness. Cheeran et al. observed MEP amplitude facilitation following cathodal TDCS-primed 1 Hz rTMS (i.e. homeostatic interaction) in subjects without the polymorphism compared to subjects with the polymorphism [56]. Later work by Mastroeni and colleagues refuted this finding [57]; yet, they employed different NIBS methodology (continuous and intermittent TBS) that might have also affected the homeostatic plasticity response.

We surmise that individual stroke characteristics also affect one's response to priming. Well-recovered individuals in chronic stages of stroke recovery may not depict deficiencies

in ipsilesional excitability or imbalances in IHI. On average, participants in this study did not demonstrate IHI imbalances (Table 3). As previously stated, the primary outcome measures in this study were differences in cortical excitability from baseline. Detecting priming responses may be difficult when ipsilesional and contralesional hemispheres display similar amounts of cortical excitability. However, previous research in healthy individuals has clearly shown priming responses and subsequent homeostatic plasticity. Other stroke-related factors like lesion size, preservation of white matter tracts, and current medication use may also impact individual responsiveness to 6 Hz priming and may contribute additional complexity in our ability to not only identify a priming response but to also distinguish priming responses. We observed no definitive ‘priming responder profile’ amongst those participants that responded as hypothesized to 6 Hz primed 1 Hz rTMS. These participants varied in stroke type (ischemic vs. hemorrhagic) and stroke extent (cortical vs. subcortical). Future research that recruits a more homogenous stroke sample may clarify discrepancies between priming responders and nonresponders.

Consistent with Bradnam et al.’s assertion that NIBS protocols in stroke are not a “one size fits all” phenomenon [52], it is also likely that priming/conditioning contralesional M1 was not the optimal therapeutic target for all participants. Future priming studies in stroke should acknowledge the array of functional connections between M1 and other sensorimotor regions that could serve as potential priming targets for M1 conditioning. Previous work in healthy individuals has shown neuroplastic change in M1 following priming stimulation to somatosensory [58], supplementary [59], and premotor [60] cortices and cerebellum [61]. These alternative priming targets may not only enhance the extent of homeostatic interactions but may also elevate consequential motor learning.

We included a secondary behavioral measure in our study since previous work has shown improvements in paretic hand function following a single rTMS session without any prior behavioral training [5,62]. The value of including a behavioral measure is also justified by a recent metaanalysis that showed significant positive effects of paretic hand and finger function following rTMS but non-significant effects for neurophysiological TMS measurements [63]. Accounting for the significant carryover and period main effects, we suspect that the improvements in Box and Block performance reflect a training effect and not a true treatment effect. The significant treatment-by-period interaction represents an imbalance in the amount of carryover between treatments. It is important to note that the gains in performance following 6 Hz and 1 Hz priming did not surpass the minimal detectable difference value of 5.5 blocks and, therefore, would not be considered true improvement from baseline even in the absence of carryover, period effects, etc.

Study Limitations

We acknowledge several limitations in our study. Aside from the small sample size that resulted in an unbalanced number of participants assigned to each treatment sequence, the mixed effects linear model did not completely negate the effects of inter-individual variability. The model calculated posttest differences from baseline for each individual prior to computing a group average of baseline differences for each posttest. Inter-individual differences in stroke characteristics, medication-use, and responses to priming and rTMS,

for example, influence the overall magnitude of baseline change. Though a single-subject design may remedy this issue, the statistical computations involved in such a design do not address potential treatment effect confounders like carryover. Indeed, we encountered significant carryover on several occasions. Though we acknowledged its detrimental effects in a crossover study design, we can also interpret carryover as a long-lasting treatment effect. Hence, a one-week washout period was insufficient. Finally, we emphasize caution when interpreting our results since we did not correct for multiple comparisons.

The design of this study featured three post-intervention assessments on subsequent days following the intervention to probe both short-term retention of effects or effects with a late onset. It is possible that a lack of multiple post-test measures following the intervention on Day 3 may have missed short-lasting effects arising 60+ minutes after treatment, for example.

One last limitation is the absence of control experiments, similar to those conducted in prior work [12,23,50] that examine the directionality of MEP amplitude and/or intracortical excitability change following priming only. Because of varying individual responses to rTMS, the possibility exists that not all participants demonstrated the expected directional responses to 6 Hz or 1 Hz priming. Moreover, Quartarone et al. found impaired homeostatic responses in individuals with focal hand dystonia that, upon further review, did not show inhibitory after-effects following cathodal TDCS priming [64]. These results underscore the necessity of including control experiments to confirm the priming effects. Lastly, the duration of metaplasticity likely spans several minutes to hours. Posttests occurred immediately after treatment and approximately 24 and 48 hours thereafter. We may not have captured the most robust modulations of cortical excitability following treatment. However, our results (e.g. CSP measures) depict heightened effects of 6 Hz priming days after treatment. Additional research is necessary to expand upon work by Fricke et al. that examined the timecourse of metaplasticity and the timing between priming and conditioning events [21].

Conclusion

In summary, our findings demonstrate the efficacy of 6 Hz primed 1 Hz rTMS in probing homeostatic plasticity mechanisms in the stroke brain as best demonstrated by differences in ipsilesional CSP duration and SICI from baseline. In contrast to our primary hypothesis, however, 6 Hz priming was not universally superior to 1 Hz and sham 6 Hz priming types across all measures of cortical excitability and paretic hand function. Inter-individual differences in stroke characteristics and responses to priming likely contributed to these incongruities. Continued exploration of homeostatic metaplasticity in the stroke brain using NIBS is important in order to fully realize the capabilities of NIBS in stroke recovery and rehabilitation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AMP	amplitude
BCM	Bienenstock-Cooper-Munro
BDNF	brain-derived neurotrophic factor
CSP	cortical silent period
EMG	electromyography
FDI	first dorsal interosseus
ICF	intracortical facilitation
IHI	interhemispheric inhibition
M1	primary motor cortex
MEP	motor-evoked potential
NIBS	noninvasive brain stimulation
PAS	paired-associative stimulation
RMT	resting motor threshold
rTMS	repetitive transcranial magnetic stimulation
SICI	short-interval intracortical inhibition
TBS	theta-burst stimulation
TDCS	transcranial direct current stimulation

Appendix A

The following equation describes the mixed-effects linear model:

$$y_{kij} = (\beta_0 + b_k) + t_j + p_i + c_i' + \varepsilon_{kij}, \quad \text{Eq.(B.1)}$$

where y_{kij} represents the response from participant k receiving sequence i at period j . β_0 is the overall mean intercept, b_k is the random intercept for subject k , t_j is the treatment effect at period j , p_i is the period effect of sequence i , c_i' depicts carryover from the previous period, and ε_{kij} represents random error that is independent from random intercept b_k and is assumed to normally distributed [65].

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Highlights

- Significant CSP and SICI differences from baseline with active 6 Hz priming.
- Significantly greater decreases in CSP duration from baseline with 6 Hz priming vs. 1 Hz and sham 6 Hz priming.
- 6 Hz priming did not potentiate ICF or IHI differences from baseline vs. 1Hz and sham 6 Hz priming.

Figure 1. Study Schedule depicting crossover design where participants received three different repetitive transcranial magnetic stimulation (rTMS) treatments over a five-week course. Participants completed interhemispheric inhibition (IHI), short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and cortical silent period (CSP) testing in addition to a behavioral test (Box and Block).

Figure 2.

Electromyography Recordings of Motor-Evoked Potentials (MEPs). a) Resultant MEP trace (top line) following a single TMS pulse to ipsilesional primary motor cortex (M1).

Inhibition of ipsilesional M1 (top line, b) occurs when a suprathreshold TMS pulse is delivered 10 ms earlier to contralesional M1 (bottom line, b). Measuring contra-to-ipsilesional IHI is done by computing the paired-pulse (top line, b) to single-pulse (top line, a) MEP amplitude ratio. Inhibition in the ipsi-to-contralesional direction is shown in panels c and d. There is little to no change in MEP amplitude when comparing contralesional M1 MEPs following single-pulse (bottom line, c) and paired-pulse (bottom line, d) conditions when a suprathreshold conditioning pulse delivered to ipsilesional M1 (top line, d) precedes the test pulse to contralesional M1.

Figure 3.

Interhemispheric Inhibition (IHI) depicting contra-to-ipsilesional IHI (top red arrow, a) and ipsi-to-contralesional IHI (bottom red arrow, b). Values are least squares means $\pm$ standard errors for differences in bilateral paired-pulse to single-pulse motor-evoked potential amplitude ratios from baseline. Positive values indicate reduced contra-to-ipsilesional IHI from baseline. Negative values indicate increased ipsilesional-to-contralesional IHI from baseline. Open shapes represent significant within-treatment differences from baseline ($p < 0.05$). † denotes significant between-treatment differences for A vs. C ($p < 0.05$), AMP=

amplitude, Contra= contralesional, Ipsi= ipsilesional, M1= primary motor cortex, MEP= motor-evoked potential, PP= paired-pulse, SP= single-pulse

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Figure 4.

Ipsilesional Intracortical Excitability depicting short-interval intracortical inhibition (SICI, a) and intracortical facilitation (ICF, b). Values are least squares means $\pm$ standard errors for differences in unilateral paired-pulse (PP) to single-pulse (SP) motor-evoked potential amplitude ratios from baseline. Positive values indicate reduced SICI from baseline (a) or increases in ICF from baseline (b). Open shapes represent significant within-treatment differences from baseline ($p < 0.05$). AMP= amplitude, Ipsi= ipsilesional, M1= primary motor cortex, MEP= motor-evoked potential, PP= paired-pulse, SP= single-pulse

Figure 5.

Ipsilesional Cortical Silent Period (CSP). Values are least squares means $\pm$ standard errors of CSP duration difference from baseline. Negative values indicate decreased CSP duration from baseline. Open shapes denote significant within-treatment differences from baseline ($p < 0.05$). * represents significant between-treatment differences for A vs. B ($p < 0.05$). † denotes a significant between-treatment difference for A vs. C ($p < 0.05$).

Figure 6.

Box and Block. Values are least squares means $\pm$ standard errors plotted for differences from baseline in the number of blocks transferred by participants using their paretic hand. Open shapes represent significant within-treatment differences from baseline ($p < 0.05$).

Table 1

Demographic and Imaging

Participant	Sex	Age (y)	Time Post-Stroke (m)	Stroke Hemisphere	Involvement	Type	Location	Affected Side	Prior Handedness
1	M	64	118	L	C/S	I	FPL	R	R
2	M	84	106	L	C/S	I	FPL	R	R
3	F	71	29	L	S	I	PFL, DWM	R	R
4	M	72	20	L	C/S	I	CR, PL	R	R
5	M	48	16	L	S	H	T	R	R
6	F	60	13	L	S	I	VM	R	R
7	F	59	95	R	C	I	FL	L	R
8	M	63	12	R	S	H	T	L	R
9	M	67	34	L	C/S	I	CA, FPL, T	R	R
10	M	74	29	R	C/S	I	PFL, PLEC	L	R
11	M	64	159	L	S	I	Pons	R	R

CA caudate, CR corona radiata, C/S cortical/subcortical, DWM diffuse white matter, F female, FL frontal lobe, FPL frontoparietal lobe, H hemorrhagic, I ischemic, L left, M male, m months, PFL posterior frontal lobe, PL parietal lobe, PLEC posterior limb of external capsule, R right, S subcortical, T thalamus, VM ventral medulla, y years

Table 2

Participant Screening Results

Participant	Treatment Sequence	MMSE	NIHSS Pre	NIHSS Post	BDI-II	UEFM	EHI (%)
1	BCA	24	4	3	3	52	88.9
2	CAB	25	5	7	10	32	-29.4
3	ABC	28	3	3	14	46	-63.6
4	BAC	30	1	NA	3	64	100.0
5	BAC	30	3	3	1	58	-33.3
6	CBA	30	3	2	6	30	-66.7
7	ACB	28	3	3	32	64	66.7
8	ACB	29	10	7	24	12	100.0
9	CBA	18	3	3	6	44	-40.0
10	CAB	30	2	2	3	54	66.7
11	ABC	29	3	NA	11	51	-82.0

BDI-II Beck Depression Inventory Second Edition, EHI Edinburgh Handedness Inventory (prior to stroke), MMSE Mini-Mental State Examination, NA not available, NIHSS National Institutes of Health Stroke Scale, Pre pretest, Post posttest, UEFM Upper Extremity Fugl-Meyer; Negative EHI values indicate left upper- extremity preference

Table 3

Group Baseline Measurements

Measure	Week 1 (Average $\pm$ SD)	Week 3 (Average $\pm$ SD)	Week 5 (Average $\pm$ SD)
Ipsilesional M1 RMT 50 mm coil, (% of Machine Output)	56.09 $\pm$ 16.40	53.75 $\pm$ 19.17	52.90 $\pm$ 20.10
Contralesional M1 RMT 50 mm coil, (% of Machine Output)	44.41 $\pm$ 9.32	43.15 $\pm$ 11.15	44.15 $\pm$ 12.50
Ipsilesional M1 RMT 70 mm coil, (% of Machine Output)	56.00 $\pm$ 19.38	51.15 $\pm$ 17.81	51.15 $\pm$ 20.03
Ipsilesional M1 MEP Amplitude 50 mm coil, (millivolts)	0.494 $\pm$ 0.454	0.489 $\pm$ 0.367	0.486 $\pm$ 0.347
Contralesional M1 MEP Amplitude 50 mm coil, (millivolts)	0.700 $\pm$ 0.259	0.597 $\pm$ 0.258	0.698 $\pm$ 0.296
Ipsilesional M1 MEP Amplitude 70 mm coil, (millivolts)	0.528 $\pm$ 0.470	0.507 $\pm$ 0.369	0.423 $\pm$ 0.386
Ipsi-to-Contralesional Interhemispheric Inhibition (PP/SP MEP Ratio)	0.718 $\pm$ 0.283	0.791 $\pm$ 0.249	0.672 $\pm$ 0.255
Contra-to-Ipsilesional Interhemispheric Inhibition (PP/SP MEP Ratio)	0.742 $\pm$ 0.315	0.990 $\pm$ 0.384	0.862 $\pm$ 0.343
Short-Interval Intracortical Inhibition (PP/SP MEP Ratio)	0.686 $\pm$ 0.237	0.647 $\pm$ 0.248	0.627 $\pm$ 0.343
Intracortical Facilitation (PP/SP MEP Ratio)	1.386 $\pm$ 0.776	1.352 $\pm$ 0.523	1.690 $\pm$ 1.098
Cortical Silent Period (milliseconds)	228.85 $\pm$ 79.88	236.72 $\pm$ 92.34	217.16 $\pm$ 73.20
Box and Block (# of blocks)	29.06 $\pm$ 20.32	31.92 $\pm$ 21.14	33.25 $\pm$ 20.80

M1 primary motor cortex, MEP motor-evoked potential, mm millimeter, PP/SP paired-pulse to single-pulse, RMT resting motor threshold, SD standard deviation