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ORIGINAL PAPER

Both 50 and 30 Hz continuous theta burst transcranial magnetic stimulation depresses the cerebellum

Nicholas D. J. Strzalkowski[1] · Aaron D. Chau[1] · Liu Shi Gan[1] · Zelma H. T. Kiss[1]

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Abstract
The cerebellum is implicated in the pathophysiology of numerous movement disorders, which makes it an attractive target for noninvasive neurostimulation. Continuous theta burst stimulation (cTBS) can induce long lasting plastic changes in human brain; however, the efficacy of different simulation protocols has not been investigated at the cerebellum. Here, we compare a traditional 50-Hz and a modified 30-Hz cTBS protocols at modulating cerebellar activity in healthy subjects. Seventeen healthy adults participated in two testing sessions where they received either 50-Hz ($cTBS_{50}$) or 30-Hz ($cTBS_{30}$) cerebellar cTBS. Cerebellar brain inhibition (CBI), a measure of cerebello-thalamocortical pathway strength, and motor evoked potentials (MEP) were measured in the dominant first dorsal interosseous muscle before and after (up to ~ 40 min) cerebellar cTBS. Both cTBS protocols induced cerebellar depression, indicated by significant reductions in CBI ($P < 0.001$). No differences were found between protocols ($cTBS_{50}$ and $cTBS_{30}$) at any time point ($P = 0.983$). MEP amplitudes were not significantly different following either cTBS protocol ($P = 0.130$). The findings show cerebellar excitability to be equally depressed by 50-Hz and 30-Hz cTBS in heathy adults and support future work to explore the efficacy of different cerebellar cTBS protocols in movement disorder patients where cerebellar depression could provide therapeutic benefits.

Keywords Cerebellum · Transcranial magnetic stimulation · Theta burst stimulation · TMS · cTBS

Introduction

Transcranial magnetic stimulation (TMS) is a commonly used research technique and a promising clinical tool to noninvasively stimulate the brain. A TMS coil produces a magnetic field that penetrates the scalp with minimal impedance and can induce electrical currents in underlying, superficial tissue, i.e., cerebral and cerebellar cortices. These induced currents in the brain act to depolarize neurons in stimulated regions [1]. TMS not only modulates the locally stimulated neurons but can also modulate distant connected structures allowing network effects to be studied. The effects of TMS on neuronal activity depend on the number and pattern of stimulation pulses delivered to the brain. Single TMS pulses delivered to the primary motor cortex (M1) evoke motor evoked potentials (MEPs) in contralateral muscles, which provide a measure of M1 excitability. Repetitive trains of TMS pulses (rTMS) can induce excitatory and inhibitory neuroplasticity that outlast the stimulation duration [2]. The noninvasive nature and therapeutic potential of rTMS have motivated research and therapeutic applications in a variety of clinical populations including Parkinson's disease, stroke, major depression, and schizophrenia [3].

The application of rTMS in short bursts of stimuli repeated at theta frequencies (4–7 Hz) has emerged as a popular technique due to its relatively short application time (~ 3 min or less) and lasting neuroplastic aftereffects [3]. Theta burst stimulation (TBS) was first proposed by Huang and colleagues (2005), who described three different TBS protocols that each consisted of three-pulse bursts delivered at an inter-pulse interval of 50 Hz and an inter-burst interval of 5 Hz. Intermittent TBS (iTBS), a 2-s train of TBS repeated every 10 s for 190 s was

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✉ Zelma H. T. Kiss
zkiss@ucalgary.ca

[1] Department of Clinical Neurosciences, Faculty of Medicine, Hotchkiss Brain Institute, University of Calgary, Calgary, AB T2N4N1, Canada

shown to increase MEP amplitudes. Conversely, continuous TBS (cTBS), a 40-s train of uninterrupted TBS was shown to reduce MEP amplitudes. Intermediate TBS (imTBS), a 5-s train of TBS repeated every 15 s for 110 s was found to have no effect on neuronal excitability [2]. These original TBS protocols have been used in hundreds of studies [4]; however, the parameters have remained largely unchanged from the original 50-Hz protocol [2]. An exception to the 50-Hz protocol is the limited use of a modified 30-Hz cTBS protocol first shown to induce behavioral effects in the oculomotor system when applied to the frontal eye field region [5] and posterior parietal cortex [6]. Thirty-hertz cTBS was originally investigated for its capability to deliver higher intensity stimulation compared to traditional 50-Hz cTBS, which is advantageous in populations with high motor thresholds [7]. When applied to the M1, 30-Hz cTBS evokes longer lasting aftereffects (MEP suppression) with less inter-individual variability compared to 50-Hz cTBS [8]. These studies indicate that different cTBS parameters can effectively modulate cerebral cortex excitability and support additional studies to explore the efficacy of different TBS protocols across the brain regions.

The cerebellum is an attractive target for TBS because of its involvement in motor control [9, 10] and role in movement disorders [11]. The cerebellum exerts influence on motor cortex excitability through the disynaptic cerebello-thalamocortical (CTC) pathway [12], and cerebellar TMS is thought to act on Purkinje cells in the cerebellar cortex [13, 14]. When active, Purkinje cells inhibit the dentate nucleus, which leads to a disfacilitation of thalamocortical drive. Single TMS pulses to the lateral cerebellum can reduce the size of subsequent M1 evoked MEPs, and this technique is called cerebellar brain inhibition (CBI) [15]. Fifty-hertz cTBS applied to the cerebellum reduces CBI [9] and inhibits galvanic vestibular reflexes [16]. TMS-induced cerebellar inhibition has previously been shown to reduce levodopa-induced dyskinesia in Parkinson's disease (rTMS) [17] and improve cervical dystonia (cTBS) [18]. Modulating cerebellum activity through TMS has clear research and clinical applications; however, the effectiveness of different rTMS and TBS protocols in this location is unknown.

Despite the clear anatomical and functional differences between M1 and cerebellum, most studies employ the same TMS protocols on both brain regions [3, 4]. Here, we built upon a previous study where a 30-Hz cTBS protocol applied to the motor cortex was more effective at depressing M1 excitability than a 50-Hz protocol [8] and compared the efficacy of traditional 50-Hz and a modified 30-Hz cTBS protocol in modulating cerebellar activity. Maximizing the magnitude and duration of cerebellar depression would increase the usefulness of TBS as both a clinical and research tool.

Methods

Subjects

Seventeen subjects (9 male, 8 female, mean age 24, range 19–36) with no history of neurological disorders participated in the study. Subjects were screened for eligibility (Rossi et al. 2011) and gave written informed consent prior to data collection. The experimental procedures were approved by the University of Calgary Research Ethics Board and complied with the Declaration of Helsinki.

Experimental Overview

All subjects participated in two testing sessions, separated on average by 8 days (range 1–30). Subjects were seated for all procedures, with their arms resting on a pillow across their lap. In the first session, subjects were randomly assigned to receive either 50-Hz cTBS ($cTBS_{50}$) or 30-Hz cTBS ($cTBS_{30}$) to the lateral cerebellum ipsilateral to their dominant hand. The alternative cTBS protocol was tested in the second session. Cerebellar brain inhibition (CBI) and MEP amplitudes were recorded from the first dorsal interosseous (FDI) of the dominant hand before (pre) and after (post1, 4–20 min, post2, 25–40 min) cerebellar cTBS. Neuronavigated TMS (Brainsight2, Rogue Research Inc., Montreal, Canada) was used to ensure consistent coil orientation over the FDI motor cortex hotspot within and between experimental sessions. Subjects wore ear plugs during CBI testing. MEPs were recorded in the FDI using surface electromyography (EMG), with one electrode placed over the muscle belly and another over the metacarpophalangeal joint of the index finger (1×1 cm^2, Kendall H69P electrodes, Covidien, MA, USA). Resting (RMT) and active (AMT) motor thresholds were first obtained using the built-in EMG system of Brainsight2 (digitized at 3 KHz, gain of 2500). A Bortec AMT-8 EMG system (gain of 1000, band pass filtered between 10 and 1000 Hz; Bortec Biomedical Ltd., Calgary, AB, Canada) with Clampex software (digitized at 10 kHz; Clampex 10.6, Molecular Devices, San Jose, CA, USA) was then used to collect CBI and MEP data pre and post cerebellar cTBS.

Cerebellar Continuous Theta Burst Stimulation

A 70-mm double Airfilm coil and Super Rapid[2] Plus[1] TMS stimulator (The Magstim Company Ltd., Whitland, UK) were used to deliver cTBS over the lateral cerebellum ipsilateral to the dominant hand, 3 cm lateral to the inion on the line joining the inion and the external auditory meatus. The coil was positioned tangentially to the head, with the handle pointing upwards [9]. In both cTBS protocols, 600 pulses were delivered at 80% active motor threshold (AMT). AMT was determined in each test session as the minimum stimulation intensity that could evoke an MEP ≥200 μV in five of ten trials as the subjects held a 10% maximum contraction of their FDI [2]. $cTBS_{50}$ involved three-pulse bursts at 50 Hz with bursts repeated at 5 Hz. In contrast, $cTBS_{30}$ involved three-pulse burst at 30 Hz with burst repeated at 6 Hz [8] (Fig. 1).

Cerebellar Brain Inhibition Protocol

CBI was conducted with two TMS coils in a paired-pulse paradigm. Conditioning stimuli (CS) were delivered to the lateral cerebellum with a 110-mm double-cone coil and Magstim BiStim[2] stimulator (The Magstim Company Ltd., Whitland, UK); the most efficient coil design at eliciting CBI [19]. The double-cone coil was centered over the lateral cerebellum ipsilateral to the dominant hand, 3 cm lateral to the inion on the line joining the inion and the external auditory meatus [15]. A figure-of-eight coil (D70[2], MagStim BiStim[2] stimulator, Magstim Company, UK) was used to deliver test stimuli (TS) to the FDI M1 motor hotspot. The TS coil was placed tangential to the scalp, orientated to have the lowest FDI MEP thresholds, with the handle oriented posterior and approximately 45° lateral from the midline (Fig. 2a). FDI MEPs were recorded in response to the TS alone and with the CS delivered before the TS at three inter-stimulus intervals (ISI: 3, 5, 7 ms). CBI is expressed as the ratio of conditioned (3, 5, and 7 ms ISI) to unconditioned (TS alone) MEP peak-to-peak amplitudes (Fig. 2b). Under normal conditions (i.e., pre cTBS), the cerebellar CS acts to inhibit the M1, and conditioned MEPs are expected to be smaller compared to unconditioned MEPs (smaller ratio of conditioned to unconditioned MEP amplitudes) [20]. CS intensity was set to 100% of 50-μV RMT; and three TS intensities were tested: 90%, 100%, and 110% of 500-μV RMT. Both 50-μV and 500-μV RMTs (RMT_{50} and RMT_{500}) were measured at the FDI hotspot using the figure-of-eight coil. At each TS intensity-ISI combination (including TS-alone), 10 MEPs were recorded pre, and 20 MEPs were recorded post cerebellar cTBS. MEPs were recorded in blocks of eight trials at a single TS intensity. Within a block, two trials at each ISI (including TS-alone) were randomly delivered every 6 s. Blocks were randomly delivered in sets of three (one of each TS intensity), and five sets were delivered pre, post1, and post2 (Fig. 1). This method allows for CBI and MEPs at each TS intensity to be investigated at similar time points post cTBS.

Data Analysis

CBI and MEP data were analyzed using a custom MATLAB script (MATLAB R2017, The MathWorks, Inc., Natick, USA). The effect of cTBS protocol on cerebellar function was quantified by comparing CBI-induced changes in MEP amplitudes pre and post cTBS. Trials that did not evoke an

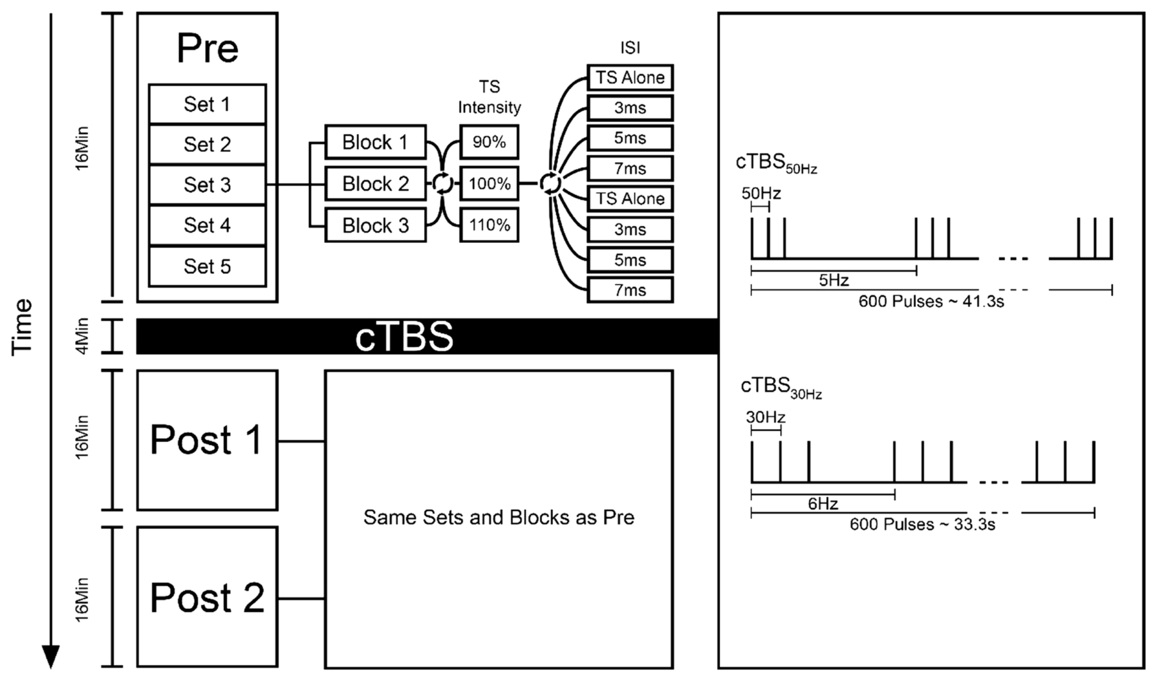

Fig. 1 Experimental design. Cerebellar brain inhibition (CBI) was measured before (pre) and after (post 1 and post 2) continuous theta burst stimulation (cTBS) was applied to the lateral cerebellum using either $cTBS_{50}$ or $cTBS_{30}$ stimulation protocols. The inhibitory effects of $cTBS_{50}$ and $cTBS_{30}$ were measured on separate days in the same subjects. CBI was measured in five sets of three blocks, in which three test stimulation intensities (TS intensity 90%, 100%, and 110% of resting motor threshold) were randomly tested pre, post 1, and post 2. Four different inter-stimulus interval trials (ISI; TS alone, 3 ms, 5 ms, 7 ms) were randomly tested twice per block. The TS intensity-ISI combination that yielded the greatest CBI pre was determined for each subject and used in pre-post comparisons

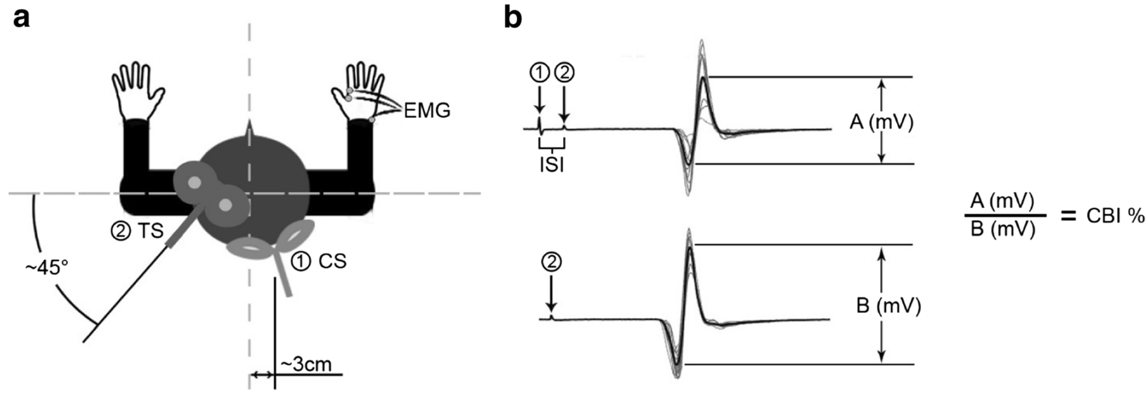

Fig. 2 Cerebellar brain inhibition (CBI). **a** Subject set up. A double cone coil (① light gray) delivered a conditioning stimulus (CS) to the cerebellum 3 cm lateral to the inion, ipsilateral to the dominant hand. A figure-of-eight coil (② dark gray) delivered a test stimulus (TS) to the contralateral first dorsal interosseous (FDI) primary motor cortex hotspot. Motor evoked potentials (MEP) were recorded using surface electromyography (EMG) from the dominant FDI. **b** Example of ten MEPs (gray) and their average (black). CBI is the ratio between conditioned MEPs where the TS is preceded by a CS shown here at a 5-ms inter-stimulus interval (ISI) and the unconditioned MEPs where the TS delivered alone

MEP were excluded from MEP averages (8 trials, only occurred using TS of 90% in 3 subjects). Data were excluded from analysis if > 2 trials were removed within the same TS intensity-ISI combination either pre, post1, or post2. The remaining 8 to 10 MEPs within the pre, post1, and post2 sets were averaged at each TS intensity-ISI combination (Fig. 1). To investigate the time course of cTBS aftereffects, block averages (average of 2 MEPs within each block at each ISI) were calculated post cTBS. The TS intensity-ISI combination (e.g., TS 90% and ISI 5 ms) that evoked the most CBI pre was determined for each subject. This optimum TS intensity-ISI combination was used to compile pre and post data for each subject. In addition to measurements of CBI, the peak-to-peak MEP amplitudes at each TS intensity were averaged for the TS-alone trials for each subject. MEP amplitude was compared pre and post cTBS.

Statistical analysis was performed using SPSS (IBM SPSS Statistics, version 24.0; Armonk, NY, USA). A linear mixed model analysis was used to compare CBI data across time (pre, post1, and post2) and cTBS condition ($cTBS_{50}$ and $cTBS_{30}$). The best-fitting covariance structure for the residuals was an autoregressive structure. Diagonal, scaled identity, compound symmetry, and unstructured covariance structures were also tested, but none showed an improved fit of the model. Significant main effects were followed up with pairwise comparisons with Bonferroni adjustments for multiple comparisons. A three-way repeated measures analysis of variance (ANOVA) with factors time (pre, post1, and post2), TS intensity (90%, 100%, and 110%), and cTBS protocol ($cTBS_{50}$ and $cTBS_{30}$) were conducted on the unconditioned MEP data. A Greenhouse-Geisser correction was applied to comparison of intensity in those cases which violated Mauchley's test of sphericity. Figures were made using Prism5 (GraphPad Prism version 5.0c for Mac OS X, San Diego CA).

Results

The TMS intensities used for the TS, CS, and cTBS (500 µV and 50 µV RMT, and 200-µV AMT) were similar (within 1% of stimulator output) between days (cTBS protocols) (Table 1). One subject was excluded from analysis because they had pre CBI values > 0.9 on both days, indicating that they were a nonresponder to CBI. Four other subjects had pre CBI > 0.9 on one of the testing days (50 Hz or 30 Hz), and their data for the corresponding stimulation protocol were excluded on these occasions. With nonresponders removed, data from 14 subjects were analyzed for each cTBS protocol. TS intensity set to 90% RMT_{500} was found to be the best at evoking CBI in 9 of 14 subject pre $cTBS_{50}$ and in 6 of 14 subjects pre $cTBS_{30}$, making it slightly better than 100% or 110% RMT_{500} at evoking CBI. Both 5 ms and 7 ms ISI durations were equally effective in evoking CBI.

Cerebellum excitability was reduced by both $cTBS_{50}$ and $cTBS_{30}$; however, a statistical difference between protocols was not observed. The linear mixed model analysis with repeated factors of: time (pre, post1, and post2) and cTBS protocol ($cTBS_{50}$ and $cTBS_{30}$) showed a significant main effect

Table 1 Resting (RMT) and active (AMT) motor thresholds used to select stimulator outputs for the CBI and cTBS protocols. RMT was determined with a figure-of-eight coil and AMT with an Airfilm coil. Test pulses were delivered at 90%, 100%, and 110% of the 500-µV RMT, conditioning pulses were delivered at 100% of 50-µV RMT, and cTBS was delivered at 80% of 200-µV AMT (10% maximum contraction). Values indicate percentages of maximum stimulator output

	500-µV RMT		50-µV RMT		200-µV AMT	
Session	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
$cTBS_{50}$	55.4 (9.9)	29–67	42.2 (5.5)	28–51	49.5 (5.2)	37–57
$cTBS_{30}$	54.4 (8.8)	31–67	41.2 (5.4)	27–49	48.9 (5.2)	37–57

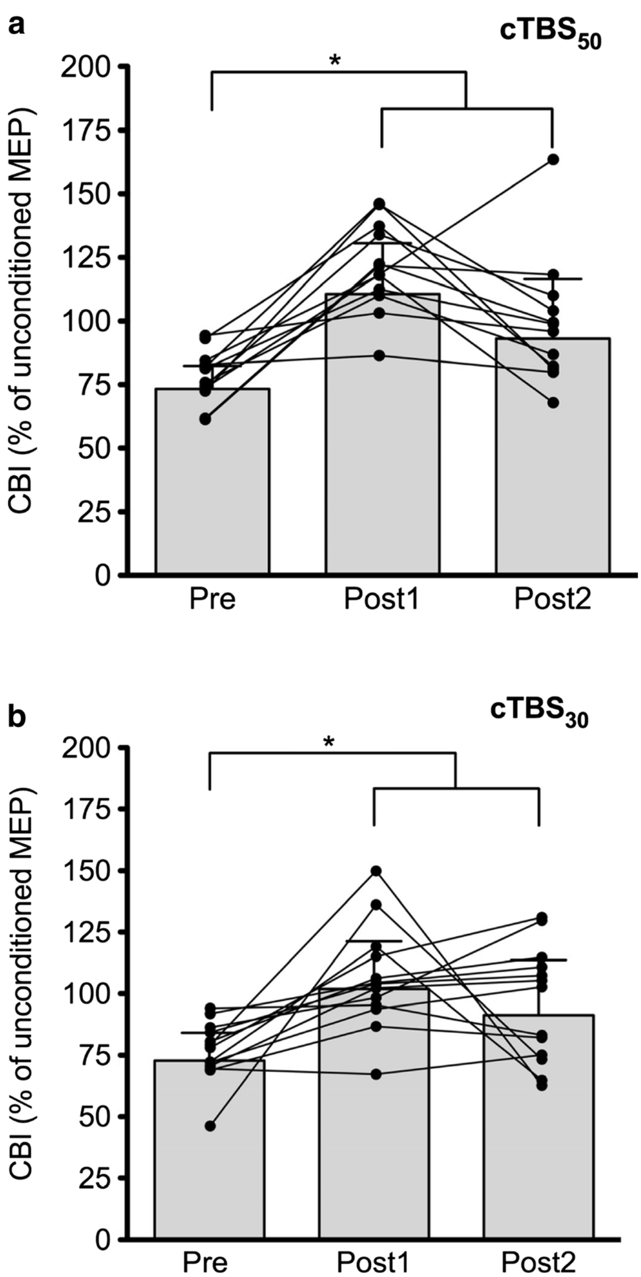

Fig. 3 Cerebellar brain inhibition (CBI), before (pre) and ~3–20 min after (post1) and ~23–40 min after (post2) 50-Hz cTBS (**a**) and 30-Hz cTBS (**b**) applied to the lateral cerebellum. Lower values indicate greater cerebellar inhibition, while values close to 100% indicate no difference between unconditioned and conditioned motor evoked potentials (MEP). Circles and lines present individual subject data, and group averages are shown by gray bars (mean, SD). CBI was significantly reduced (higher values) post1 ($P < 0.001$) and post2 ($P = 0.002$) compared to pre following both 50-Hz and 30-Hz protocols. No differences were found between post1 and post2 or between cTBS protocols at any time point ($P > 0.05$). * indicates significant difference ($P < 0.05$)

of CBI for time ($F_{2,\ 38.597} = 35.903$, $P < 0.001$), but not for cTBS protocol ($cTBS_{50}$ and $cTBS_{30}$) ($F_{1,\ 36.120} = 0.906$, $P = 0.348$) or an interaction effect ($F_{2,\ 38.597} = 0.525$, $P = 0.596$) (Fig. 3). Pairwise comparisons across time points with Bonferroni adjustment found CBI post1 ($P < 0.001$) and post2 ($P = 0.002$) to be significantly reduced (larger conditioned/unconditioned MEP ratio) compared to pre. Post1 was not found to be significantly different from post2 ($P = 0.060$).

To gain a more detailed look at how CBI measures change following cTBS over time, a linear mixed model analysis was used to investigate main and interaction effects of time and cTBS protocol on CBI with the post cTBS data separated by block of time (1–10, ~4 min each). Similar to the grouped post1 and post2 analyses, a significant main effect for time ($F_{10,\ 49.227} = 9.962$, $P < 0.001$) was obtained, but not for cTBS protocol ($F_{1,\ 89.593} < 0.001$, $P = 0.983$) nor for an interaction effect ($F_{10,\ 49.070} = 1.080$, $P = 0.3.95$) (Fig. 4). Pairwise comparisons across time points with a Bonferroni adjustment found CBI pre to be significantly greater than post cTBS block 1 ($P < 0.001$), block 2 ($P = 0.016$), and block 9 ($P = 0.007$).

Unconditioned MEP amplitudes did not change between time (pre, post1, and post2) ($F_{2,\ 13} = 2.397$, $P = 0.130$) or cTBS conditions ($cTBS_{50}$ and $cTBS_{30}$) ($F_{1,\ 14} = 0.285$, $P = 0.602$); however, MEP amplitude increased with TS intensity ($F_{2,\ 13} = 11.979$, $P = 0.001$). Pairwise comparisons across TS intensity with a Bonferroni adjustment found MEP amplitudes at 110% to be significantly larger than 100% ($P = 0.022$) and 90% ($P = 0.001$), and MEP amplitudes at 100% to be significantly larger than at 90% ($P = 0.001$).

Discussion

In summary, both 50-Hz and 30-Hz cTBS can depress cerebellar activity, as evidenced by reduced CBI. Both protocols were equally effective at reducing CBI, a finding that is in contrast to the results of Goldsworthy et al. 2012 who found a 30-Hz cTBS protocol to evoke greater, less variable, and longer lasting depression than a 50-Hz protocol when applied to M1. Therefore, the cerebellum responds differently than M1 to TMS stimulation, and further research is needed to investigate if a more effective cTBS protocol exists for cerebellum. The 50-Hz and 30-Hz cTBS protocols were selected because these are the two most common patterns currently tested in humans. This is the first study to compare cTBS protocols at the cerebellum and indicates that additional studies are needed to explore cTBS mechanisms and additional stimulation patterns.

Comparison between $cTBS_{50}$ and $cTBS_{30}$

Theta burst stimulation originated from in vitro animal studies that demonstrated high-frequency theta burst electrical stimulations induced long-term potentiation (LTP) and long-term depression (LTD) [21]. This stimulation pattern was adopted in the development of TBS at the M1, whose influence on neuronal excitability resembles LTP and LTD [2, 22]. *N*-methyl-D-aspartate (NMDA) receptors and calcium (Ca^{2+})

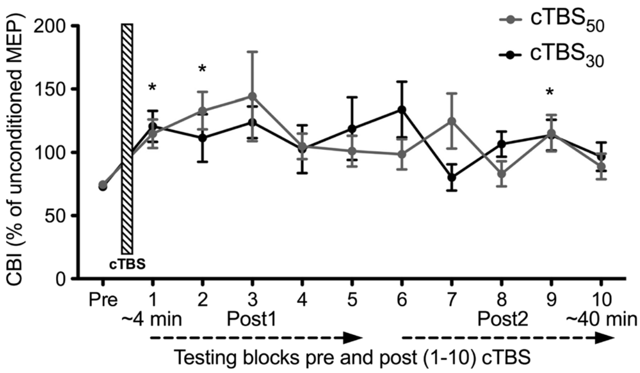

Fig. 4 Cerebellar brain inhibition (CBI; mean, SE) before (pre) and after (~ 4–40 min, blocks 1–10) $cTBS_{50}$ (gray) and $cTBS_{30}$ (black). CBI was significantly reduced (shown as larger %) at blocks 1, 2, and 9 compared to pre, which indicates depression of the cerebello-thalamocortical (CTC) pathway. No differences were found at any time point between $cTBS_{50}$ and $cTBS_{30}$. * indicates significantly higher CBI relative to pre, $P < 0.05$

channels are the most likely candidates for mediating TBS aftereffects [3, 22]. An NMDA receptor antagonist, memantine, has been shown to block the inhibitory effects of cTBS and the facilitatory effects of iTBS at the motor cortex [23]. The cTBS stimulation pattern is speculated to increase intracellular Ca^{2+} concentrations, which then evokes a cascade of inhibitory factors that reduce the number and responsiveness of postsynaptic receptors [22]. Similar studies investigating the mechanisms of TBS at the cerebellum have not been reported; however, high-frequency electrical stimulation in rat cerebellum slices has shown LTD between Purkinje cell and the deep cerebellar nuclei [24]. Future studies are needed to fully understand TBS mechanisms at the cerebellum, but similar LTD-like responses reported at the M1 likely contribute to the observed effects at the cerebellum.

In the present study, cerebellar cTBS inhibited the CTC pathway for approximately 30 min; however, significant difference between the cTBS protocols tested were not observed. This is in contrast to a previous report which found 30-Hz cTBS to evoke longer lasting aftereffects (MEP suppression) with less inter-individual variability compared to the 50-Hz cTBS at the M1 [8]. Following M1 50-Hz cTBS, MEP suppression occurred for up to 10 min, while MEP suppression persisted to 30 min following 30-Hz cTBS. The underlying neurological mechanisms for why 30-Hz cTBS may be more effective than 50-Hz cTBS at the M1 and why in the present study cerebellar cTBS appears frequency independent are not clear.

The $cTBS_{50}$ and $cTBS_{30}$ protocols differ in two aspects, the inter-pulse frequency (50 vs 30 Hz) and the inter-burst frequency (5 vs 6 Hz). These differences are noteworthy because the temporal pattern of stimulation is known to influence the aftereffects [2]. Assuming LTP- and LTD-like mechanisms are responsible for the cTBS aftereffects, concentration and rate of change of intracellular Ca^{2+} play a major role to the extent of these aftereffects [22]. It is possible that the higher inter-burst frequency of the 30-Hz cTBS protocol has a more pronounced effect on LTD Ca^{2+} mechanisms than the 50-Hz cTBS protocol, which can help explain the greater depressive effects of the 30-Hz protocol seen at the M1 [8]. Higher inter-burst and/or inter-pulse intervals may be needed to see significant differences at the cerebellum.

Cerebellar Brain Inhibition

CBI is commonly used to measure the magnitude of inhibition that the cerebellum exerts over the M1 [14]. The cerebellum conditioning stimulus is thought to activate inhibitory Purkinje cells in the cerebellar cortex, which suppresses the excitatory output of the deep cerebellar nuclei and thalamus [25]. The resulting decrease in excitatory thalamocortical drive suppresses M1 excitability evidenced by reduced MEP amplitudes. Larger reductions in conditioned MEPs suggest greater activity in the CTC pathway. CBI is reduced in Parkinson's disease [26, 27] and dystonia patients [28] compared to healthy controls. Improving CTC pathway integrity may have therapeutic benefits for movement disorder patients, and CBI measurements may prove a valuable assessment tool.

In the present study, CBI was used as the dependent variable, providing a measure of the excitability of the CTC pathway. For this purpose, we implemented different TS intensities and ISIs to maximize CBI for each participant on each day. We found average pre cTBS CBI to be 0.73, which is similar to previous studies (~ 0.65, Popa et al. 2010; ~ 0.80, Koch et al. 2008; and ~ 0.70, Carrillo et al. 2013). CBI in the present study was found to be subtle, and no combination of single TS intensity-ISI demonstrated convincing CBI in this subject group. Because our primary aim was to compare cTBS protocols on CBI, subject specific TS Intensity-ISI combinations were employed in the present study to assist in the detection of cTBS (50 vs 30 Hz) effects on CTC pathway integrity. This approach may have exaggerated the inhibitory effect compared to previous studies that employed the same CBI protocol across subjects. It is unclear why greater CBI was not found at a single TS intensity-ISI when averaged across subjects as reported by others [9]; however, we suspect that if larger CS amplitudes were applied [29], we would have evoked great CBI.

No Change in Motor Evoked Potential Amplitude

There are conflicting reports regarding the influence of cerebellar TBS on MEP amplitude. Cerebellar cTBS is thought to depress the membrane excitability of Purkinje cells, and therefore increase the output of the dentate nucleus which then leads to cortical excitation [13]. In agreement with this model, MEP amplitudes have been shown to increase following inhibitory cerebellar 1-Hz rTMS [30]. It is therefore surprising that unconditioned MEP amplitudes at 90%, 100%, and 110% RMT_{500} were not significantly modified by cTBS in the present or in a previous study [9]. Furthermore, additional studies have reported MEP amplitudes to decrease following cerebellar cTBS [13, 27, 31, 32]. cTBS is delivered at a relatively low stimulation intensity (80% AMT), which may help explain the heterogeneous M1 excitability aftereffects reported in the literature. cTBS likely only induces changes in Purkinje cells with the lowest thresholds and their downstream M1 neurons [13]. In the present study, the absence of a change in unconditioned MEP amplitude suggests that the excitability of these downstream M1 neurons was not meaningfully modulated by cTBS. In contrast, the relatively high amplitude CS delivered during CBI leads to a measurable decrease in M1 excitability due to the evoked Purkinje cell volley, which is reduced following cerebellar cTBS. Cerebellar TMS, be it cTBS or CBI, will influence a subset of associated M1 neurons. The observation in the present study that TS at 90% RMT_{500} produced the best CBI in the majority of subjects suggests that lower threshold neurons in the M1 are predominantly influenced by cerebellum TMS. It is clear that the mechanisms of cerebellar cTBS are poorly understood, and multiple pathways may be contributing to the aftereffects [13]. Future work is needed to better understand how TMS intensity dictates the modulation of neuronal excitability.

Clinical Significance

We are only in the infancy of TBS research; however, there is already interest and demand for clinical TBS applications [3]. The clinical goal of TMS as a therapeutic intervention is to improve brain functions by modulating neuronal excitability. TMS interventions should therefore be quick and noninvasive; requirements met by TBS.

The cerebellum is an attractive target for TBS investigations because of its accessibility and involvement in many movement disorders. The cerebellum is thought to be hyperactive in Parkinson's disease [11, 33] and dystonia [18], and normalizing CTC pathway activity may improve clinical symptoms [17, 25, 26]. Parkinson patients have deficient CTC inhibitory interactions that are not restored by standard dopaminergic medication [27]. Cerebellar cTBS and rTMS have been shown to have some clinical benefits, including reductions in levodopa-induced dyskinesia [17] and resting tremor [34] in Parkinson's disease. Previous studies have only investigated the efficacy of rTMS or 50-Hz cTBS, and the efficacy of alternative parameters is unknown. The present data indicate that 30-Hz cerebellar cTBS can also effectively modulate activity in healthy adults. Future studies are needed to explore the efficacy of 30 Hz and additional cTBS protocols in clinical populations at the cerebellum and other brain sites.

Limitations and Recommendations

TMS is relatively quick and simple to deliver; however, responses are often variable between people and factors contributing to response amplitude and duration that are poorly understood [35, 36]. At M1, intrinsic factors in the recruitment of TMS indirect waves (I-waves) can partly explain inter-individual variability in TBS aftereffects [37]. It is unclear if a similar measurement (direct-indirect wave latency) can be used to predict cerebellar TBS responders and nonresponders. A test to pre-screen individual susceptibility to TMS-induced neuroplasticity would have great research and clinical value. The duration of cTBS effects and when to appropriately retest subjects are an outstanding question. Two weeks of bilateral cTBS was found to have a modest clinical improvement in cervical dystonia patients when measured ~2 days but not 2 weeks post cTBS [18]. This suggests a cumulative influence of repeated cTBS with effects that can last days. The research and therapeutic value of cTBS would be increased by longer aftereffects; however, cTBS depression is only reported out to 30 to 60 min in the motor cortex [2, 9] and cerebellum [27] after single application, which is in agreement with the present findings.

CBI is an indirect measure of cerebellum excitability and may not fully capture the cTBS aftereffects on cerebellum or M1 excitability. In the present study, we optimized CBI TS intensity and ISI to account for inter-individual variability and found no differences in between $cTBS_{50}$ and $cTBS_{30}$ at decreasing CBI. A more sensitive measure of cerebellum activity (i.e., fMRI) may have been able to detect differences between cTBS protocols; however, the functional significance of a subtle difference, if present, would be questionable. A consequence of optimizing the CBI parameters in the present study is a reduced number of MEPs per block. The data presented in Fig. 4 is an average of two MEPs per 4-min window and may only provide a rough approximation of CBI changes over these time windows.

One subject in the present study did not show CBI with any TS-ISI combination at any testing set, and CBI was absent in four other subjects on one of the two testing days. It is unclear what factors contribute to a subject's susceptibility to demonstrating CBI. In some cases, the CS was likely insufficient to evoke a measurable inhibitory volley from the cerebellar cortex. A recent study found a CS of 60% maximum stimulator output evoked reliable CBI [29]. Whereas in our study, CS was normalized to 100% of a 50-μV RMT that resulted in CS amplitudes ranging from 38 to 54% of maximum stimulator output. It is likely that with higher CS amplitudes, larger CBI would have been observed across subjects and TS-ISI combinations.

In the present study, a TS intensity at 90% RMT_{500} generally showed the best CBI pre cTBS. We suggest that this may be due to the low threshold afferent pathways activated by the cerebellum CS. Using even lower TS intensities and higher CS intensity may improve the magnitude and usefulness of CBI as a measure of CTC inhibitory efficacy. Similarly, a higher cTBS intensity would likely evoke greater cerebellar inhibition. The 80% AMT intensity used in the present study was well tolerated by all subjects and was based on previous work at the M1 [8]. However, the cerebellar cortex is deeper than the M1 and may be better targeted with higher intensity stimulation.

Conclusions

Both 50-Hz and 30-Hz cTBS can equally suppress CTC pathway activity. Suppressive effects were most pronounced in the first 15 min, but reduced cerebellum activity may persist up to 30 min. These findings support further investigations to explore how additional changes in cTBS stimulation parameters (inter-pulse and inter-burst intervals) impact neuroplasticity induction in the cerebellum, in healthy and diseased populations. Optimizing TBS protocols at the cerebellum is a critical step prior to the development of clinical applications.

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Compliance with Ethical Standards

The experimental procedures were approved by the University of Calgary Research Ethics Board and complied with the Declaration of Helsinki.

Conflict of Interest The authors declare that they have no conflicts of interest.

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