



The Impact of Stimulation Intensity and Coil Type on Reliability and Tolerability of Cerebellar Brain Inhibition (CBI) via Dual-Coil TMS

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Abstract

Cerebellar brain inhibition (CBI) describes the inhibitory tone the cerebellum exerts on the primary motor cortex (M1). CBI can be indexed via a dual-coil transcranial magnetic stimulation protocol, whereby a conditioning stimulus (CS) is delivered to the cerebellum in advance of a test stimulus (TS) to M1. The CS is typically delivered at intensities over 60% maximum stimulus output (MSO) via a double-cone coil. This is reportedly uncomfortable for participants, reducing the reliability and validity of outcomes. This feasibility study investigates the reliability and tolerability of eliciting CBI across a range of CS intensities using both a double-cone and high-powered figure-of-8 coil, the D70². It was expected that the double-cone coil would elicit CBI at intensities upwards of 60%MSO. The range for the D70² coil was exploratory. The double-cone coil was expected to be less tolerable than the D70² coil. CBI was assessed in 13 participants (25.92 ± 5.42 years, six female) using each coil (randomized) over intensities 40, 50, 60, 70, 80%MSO. Tolerability was assessed via visual analog scales. Comparisons across intensities and tolerability were assessed non-parametrically and via a linear model. The double-cone coil elicited CBI at intensities 60, 70, and 80%MSO ($p < .05$), with suppression elicited at 60%MSO not significantly different to that at higher intensities. CBI was not reliably elicited by the D70² coil at any intensity. The double-cone coil was significantly less tolerable than the D70². A CS of 60%MSO with a double-cone coil provides a balance between the reliability and tolerability of CBI.

Keywords Transcranial magnetic stimulation · Cerebellar brain inhibition · Cerebellum · M1 · Double-cone coil · D70² coil · Tolerability

Introduction

The cerebellum exerts an inhibitory tone on cortical motor regions via the cerebellarthalamicocortical tract [1–4]. This phenomenon, termed cerebellar brain inhibition (CBI), is related to various aspects of motor control, including adaptive learning and movement initiation [5–8]. The tract originates in the cerebellum, where Purkinje cells (PCs) make up the main output pathway of the cerebellar cortex

[2]. PCs form inhibitory synapses with cells in the deep cerebellar nuclei, where information is subsequently relayed to the cortex via the thalamus [2, 4] (Fig. 1).

CBI has been assessed directly via non-invasive techniques such as transcranial magnetic stimulation (TMS) [8–14]. TMS uses principles of electromagnetic induction to induce neuronal depolarization via a plastic-coated metallic coil that is placed against the scalp [15, 16]. Coils range in size and shape. Flat coils, such as the ‘figure-of-8’ coil (Fig. 2), are often used to target specific cortical regions as they are focal, but the generated peak field can only reach relatively superficial sites [17, 18]. In contrast, angled ‘double cone’ (DC) coils (Fig. 3) have an extended range. This makes them suitable for targeting deeper structures including the cerebellum; however, the induced field is also broader and therefore less focal [17, 18].

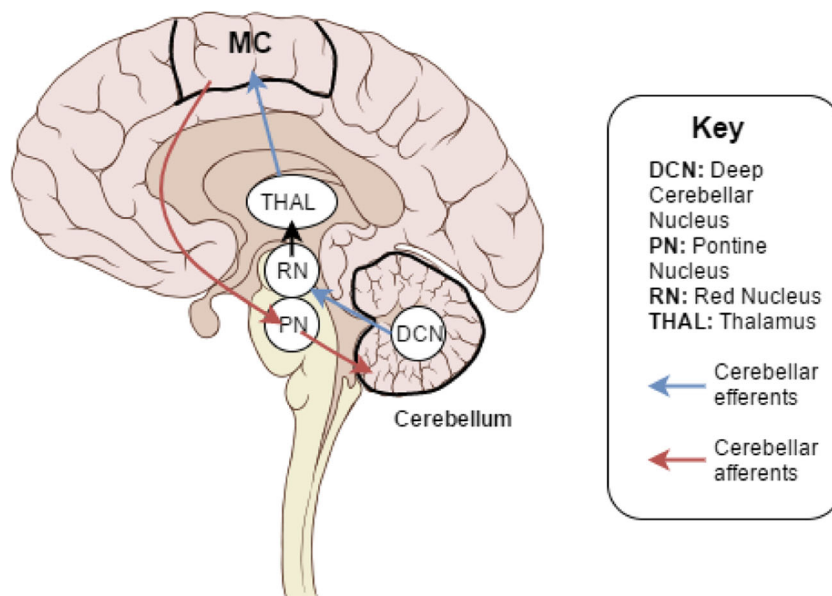
The assessment of CBI via TMS was first detailed by Ugawa, Uesaka [11]. Here, using a dual-coil protocol (Fig. 4), the amplitude of motor evoked potentials (MEPs) was

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Fig. 1 The cerebellothalamocortical tract (blue) and corticopontocerebellar tract (red). Cerebellar efferents travel from the deep cerebellar nuclei to the thalamus, reaching layer IV of the cortex. Efferents from the cortex reach the cerebellum via the pontine nucleus of the Pons



significantly decreased when a test stimulus (TS) delivered to the primary motor cortex (M1) with a flat coil was preceded (by 5–8 ms) by a conditioning stimulus (CS) to the posterior cerebellum via a DC coil [11]. This reduction in MEP was believed to be due to activation of PCs in the cerebellar cortex inhibiting cells in the deep cerebellar nuclei, and hence causing disfacilitation of neurons in cortical layers I, III, V, and VI [10, 19, 20]. However, the cerebellum lies close to neurons in the corticospinal tract (CST), a bundle of descending nerve fibers running from the cortex to motor neurons in the brainstem and spinal cord. It is possible that the coil used for cerebellar stimulation may also stimulate the CST, leading to an antidromic volley that has the potential to suppress motor cortical neurons [21].

To avoid antidromic effects from spinal structures interfering with the test pulse, the ‘active motor threshold’ (AMT)

from a direct pulse to the posterior fossa may be determined [11, 21, 22]. The cerebellar conditioning pulse should then be applied laterally at least 5–20% below threshold to ensure that suppression is due primarily to activation of the cerebellar thalamocortical tract [21, 23]. The majority of CBI studies stimulate the cerebellum at 5% maximum stimulator output (MSO) below AMT [8, 10, 24–32]. This typically requires intensities upwards of 60% MSO [21].

Unfortunately, TMS applied via a DC coil for CBI assessment is reportedly uncomfortable for participants due to the sensation of the stimulus on the scalp, in conjunction with the activation of facial and neck muscles [13, 14, 33, 34]. Not surprisingly, participant drop-outs due to stimulus discomfort have been reported [26, 35], and it is difficult to implement this protocol in vulnerable populations (e.g., autism, schizophrenia) despite the apparent cerebellar pathophysiology. To improve the tolerability of sessions, some studies lower the number of trials [14, 36]. Thus, side effects from the DC coil pulse have the potential to reduce statistical power and increase trial variance from factors such as reduced trial number,



Fig. 2 Magstim 70-mm figure-of-8 coil (Image sourced from: <https://www.magstim.com/product/6/double-70mm-alpha-coil>, 15/5/17)

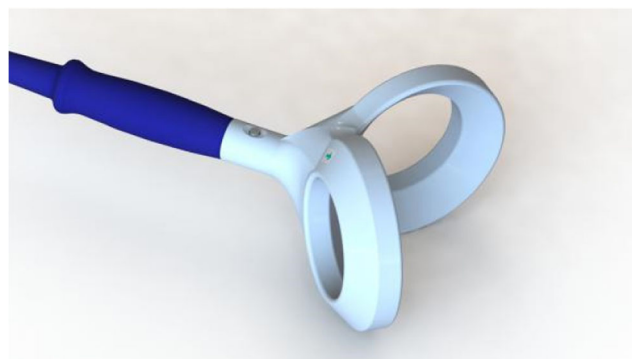


Fig. 3 Magstim 110-mm double-cone coil (Image sourced from: <https://www.magstim.com/product/16/110mm-double-cone-coil>, 15/5/17)

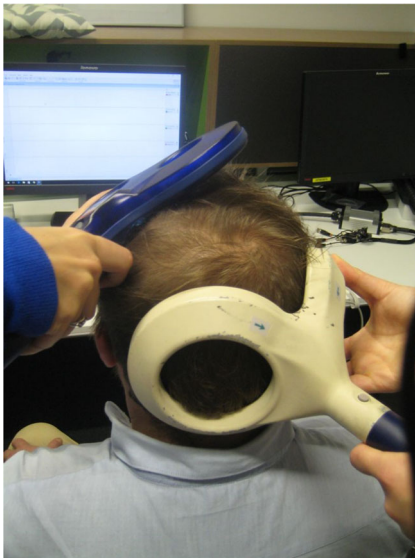


Fig. 4 TMS configuration for eliciting CBI, using double-cone coil for the CS and figure-of-8 coil for the TS

drop-outs, and muscle artefacts. Ideally, stimuli should be delivered at as low an intensity as will reliably induce CBI to increase the tolerability of trials, avoid participant drop-out, and reduce artefacts from the CS. Additionally, a factor which may counteract the depth benefits of the DC coil is that the angled design sometimes prevents the center of the coil from sitting flush against the participant's scalp. This has the potential to reduce the effects of the stimulus, as it must travel even further to reach the target site.

Flatter coil models reportedly increase trial tolerability [13] and sit flush against the scalp. Flat coils used have been used for the CS in some studies [14, 37–39]; however, the reported average depth of cerebellar gray matter is roughly 14.6–14.7 mm from the scalp surface, while the depth of the cerebellar hand representations in lobules V and VIII is in the order of 33 mm [13]. While this is within reach of the 20–30-mm peak range at 50% output, or 50–60 mm at 100% output of the DC coil, it likely lies outside the range of the figure-of-8 coil, which has been measured to be ~30 mm at 100% peak output [13, 37]. This suggests that these flat coils may not sufficiently activate PCs in the cerebellar cortex to trigger CBI when applied at lower intensities, or only do so in a subset of trials and/or participants. Recently, higher-powered figure-of-8 coils have been developed such as the D70² (The Magstim Company, Whitland, UK), which attributes its 25% increased power over previous models to increased focality (The Magstim Company, Whitland, UK). Hence, the D70² coil may activate cerebellar structures with greater reliability. Currently, no studies have investigated the reliability of the D70² in eliciting CBI when used for the CS.

In order to gauge the range over which CBI can be elicited in healthy adult participants, this feasibility study tested CBI over a range of CS intensities using both a

DC and D70² coil. The tolerability of each coil was also assessed via a series of visual analog scales (VAS), and instances when the DC coil did not sit flush against the participant's scalp were noted. We specifically assessed at which intensities CBI was reliably elicited in each coil. It was hypothesized that the DC coil would reliably elicit CBI at intensities 60%MSO and over, while the range over which the D70² coil reliably elicited CBI was exploratory. It was further hypothesized that the tolerability of each coil would decrease with increasing stimulus intensity, and the DC coil would be less tolerable than the D70².

Method

Subjects

Fourteen healthy adults (six female; mean age = 25.92 years, SD = 5.42) provided written informed consent for participation, receiving a reimbursement of AUD\$20. Due to the lateralization of cerebellar connections with the cortex [40], all subjects were right-handed, as determined by the Edinburgh Handedness Inventory [41] (M = 16.6, SD = 3.50). Additional criteria included standard contraindications for TMS such as no history of psychiatric or neurological illness, head injury, neurosurgery, serious medical illness, medical or metal implants, movement deficits, no current neuroactive medication, and no current pregnancy. Ethical approval was granted by the Deakin University Human Research Ethics Committee.

Procedure

EMG Recording and Motor Thresholds

Subjects sat upright with EMG surface electrodes placed on the first dorsal interosseous (FDI) muscle of the right hand in a belly-tendon montage, with grounding at the ulnar styloid. The EMG signal was bandpass filtered between 0.3 and 100 Hz and digitized via a Powerlab 4/35 system (ADInstruments, Colorado Springs, USA). MEPs were recorded with LabChart8 software (ADInstruments, Colorado Springs, USA) and stored offline for analysis. Resting motor threshold was determined via a figure-of-8 TMS coil connected to a Magstim 200² (Magstim, Whitland, UK), placed over the participant's motor 'hot-spot'. The coil was oriented at an angle of 45° from the midline, with handle in a posterior-anterior position. Threshold for the TS used in CBI assessment was defined as the %MSO necessary to elicit an MEP of ~0.8 mV peak-to-peak in the relaxed muscle [29], in five out of 10 trials. Notably, TS over 1.0 mV have been found to reduce the suppressive effects of the CS [29].

TMS Protocol for CBI Assessment

CBI was assessed via a dual-coil protocol [11]. The cerebellar CS was administered via a 110-mm double-cone (DC) or D70² coil (Magstim) positioned over the right cerebellar hemisphere, 3 cm lateral and 1 cm inferior to the inion, where this location is believed to target hand motor regions of the cerebellar cortex in lobules V and VIII [13]. Coil presentation order was randomized and counterbalanced across participants within one 2.5-h session, with a 5-min break between coil sets. The TS was delivered with a 70-mm figure-of-8 coil (Magstim) over the individual's motor 'hot-spot' in contralateral M1. Coils were connected to a Magstim 200² and Bistim² (Magstim, Whitland, UK). The CS was delivered 5 ms in advance of the TS, where this interstimulus interval marks the onset of CBI, and has been commonly used in past literature [14], using intensity increments of 40, 50, 60, 70, and 80%MSO, with a 1-min rest between each set. Fifteen TS and 15 CS-TS trials were randomly delivered at each intensity, with an inter-trial interval of 5 s.

To determine if the CS had elicited antidromic corticospinal activity, or activity in the spinal nerve roots, the latency of each MEP trace was visually examined at high gain [12]. Latencies of ~18 ms are believed to be the result of corticospinal antidromic volleys, while those of ~15 ms latency correspond to cervical root activity [42]. Similarly, trials showing EMG response at latencies ~5 ms were discarded, as these effects preceded the TS [12]. Latencies of ~21 ms were assumed to correspond to activity arriving from the cerebellothalamocortical tract [42]. Using this method of assessing corticospinal activity in combination with a fixed set of stimulator intensities, as opposed to comparing intensities relative to individual thresholds of the corticospinal tract, a direct comparison of tolerability across participants at various levels of suppression could be made.

Due to variations in scalp anatomy, the convergent region of the DC coil did not always sit flush against the participant's scalp, thereby potentially reducing the range of magnetic pulse. Instances when this occurred were recorded.

Pain and Discomfort

After each intensity block, a VAS was administered to gauge participant discomfort due to the cerebellar stimulation. Here, participants responded to questions, 'How painful was the stimulation', 'How much activation did you feel in your neck and/or facial muscles during stimulation?' and 'What was your general level of discomfort due to the stimulation?'. A scale between 0 (absence) and 10 (presence) was used to quantify responses. In addition to this, confirmation was regularly sought from the participant to ensure they were able to proceed to a higher intensity level.

Data Analysis

Peak-to-peak MEPs for each trial were extracted, and visually inspected at high-gain for corticospinal involvement [12]. Traces showing peaks between 5 and 20 ms from the CS were eliminated from analysis (<1%) [12, 42]. Outliers of magnitude greater than three standard deviations from the mean in the raw data were replaced by the next extreme point that was not an outlier (winsorised, <1% total). A trial set was excluded if over half of the 15 trials within that set were missing. Data were then screened for adherence to the general linear model. However, due to violations of these assumptions, Wilcoxon signed rank tests (repeated measures) on mean MEP values were performed for each coil at each intensity to confirm that suppression was occurring following the CS. To examine coil characteristics across intensities, data were expressed as the ratio of peak-to-peak MEP amplitude following the CS to the amplitude following the unconditioned stimulus (TS) (values less than one indicate suppression). As missing data made up 13% of the final dataset, linear models using maximum likelihood estimation were used for the remaining analysis. Analyses were performed for each coil separately, with CBI ratio as DV, and stimulator intensity (40, 50, 60, 70, 80%MSO) as IVs, clustered within participant (random intercept) [43]. Planned contrasts compared CBI at adjacent or near-adjacent stimulus intensities (40 vs 50%, 50 vs 60%, 60 vs 70%, and 60 vs 80%), using a *p* value of .05, Bonferroni corrected for four comparisons. However, as the distribution of the residuals differed slightly from normal (DC coil: *W* = 0.95, *p* = 0.02; D70² coil: *W* = 0.96, *p* = 0.03), analyses were also run using Wilcoxon signed rank tests (repeated measures) for comparison.

To determine if degree of suppression was correlated with coil gap (presence of) or participant RMT, Kendall's correlation analyses were performed on the ratio data for each coil at each intensity. In order to compare the tolerability of coils across intensity levels, as data did not meet assumptions of the linear model, VAS outcomes were analyzed nonparametrically for each scale separately, with coil and intensity as IVs and score as DV. Comparisons were made, (1) across coils for each intensity and (2) across intensities (40 vs 50, 50 vs 60, 60 vs 70, and 60 vs 80) within each coil.

All analyses were performed using R-Studio (version 0.99.446, R version 3.2.1).

Results

Of the 14 participants, one dropped out of the study citing psychological discomfort from the stimulus delivered with the DC coil (relating to memories of childhood trauma from repeated knocks to the back of the head). Of the remaining 13 participants, 10 completed all trials with

the DC coil. After completing trials at 60%MSO, two participants elected to not complete trials at higher intensities, and one elected to stop after 70%MSO. All of these cited physical discomfort associated with the coil, with one citing psychological anxiety surrounding the sensation of a ‘hit’ to the back of the head. All participants completed the full intensity range with the D70² coil.

Descriptive statistics for stimulation condition at each stimulator intensity (%MSO) are shown in Table 1, and for CBI ratio in Table 2. Confirming that CBI was elicited with the DC coil, Wilcoxon signed rank tests (repeated measures) on mean MEP values showed significant suppression compared with single pulse M1 stimulation ($p < .01$, Bonferroni corrected) for all intensities but 40 %MSO (50%MSO showed a trend toward significance, $p = .01$) (Table 3). However, none of the comparisons were significant for the D70² coil.

From the linear model, an examination of CBI ratio (mean conditioned/mean test) across intensities for each coil showed that suppression at 50%MSO (mean = 0.87, SD = 0.30) was significantly greater than at 40%MSO (mean = 1.09, SD = 0.37) ($b = -0.30$, $t(41) = -3.74$, $p = .0006$). Similarly,

Table 1 Descriptive statistics for stimulator intensity (%MSO) and stimulation condition

Intensity (%MSO)/ stimulation condition	Mean	Median	SD	SEM
DC coil				
40 TS	0.96	0.82	0.50	0.14
40 CSTS	0.93	0.87	0.32	0.09
50 TS	0.89	0.76	0.49	0.14
50 CSTS	0.67	0.60	0.31	0.09
60 TS	0.99	0.97	0.46	0.13
60 CSTS	0.67	0.73	0.34	0.10
70 TS	1.01	0.73	0.67	0.20
70 CSTS	0.57	0.34	0.38	0.12
80 TS	1.19	1.02	0.80	0.25
80 CSTS	0.69	0.38	0.60	0.20
D70 ² coil				
40 TS	0.73	0.73	0.35	0.10
40 CSTS	0.64	0.60	0.35	0.10
50 TS	0.69	0.71	0.32	0.09
50 CSTS	0.64	0.57	0.35	0.10
60 TS	0.72	0.61	0.41	0.11
60 CSTS	0.68	0.59	0.37	0.10
70 TS	0.71	0.64	0.40	0.12
70 CSTS	0.73	0.63	0.46	0.13
80 TS	0.82	0.78	0.33	0.09
80 CSTS	0.79	0.62	0.52	0.15

TS test stimulus alone, CSTS conditioning stimulus preceding test stimulus

Table 2 Descriptive statistics for stimulator intensity (%MSO) and CBI ratio (conditioning stimulus preceding test stimulus/test stimulus alone)

Intensity (%MSO)	Mean	Median	SD	SEM
DC coil				
40	1.09	1.00	0.37	0.10
50	0.87	0.83	0.30	0.08
60	0.74	0.78	0.31	0.09
70	0.67	0.67	0.39	0.12
80	0.59	0.59	0.30	0.10
D70 ² coil				
40	0.82	0.83	0.23	0.07
50	0.96	1.01	0.34	0.09
60	0.99	0.92	0.35	0.10
70	1.04	1.02	0.29	0.09
80	0.96	0.93	0.38	0.11

suppression at 60%MSO (mean = 0.74, SD = 0.31) was significantly greater than at 50%MSO ($b = -0.39$, $t(41) = -3.77$, $p = .0005$). However, effects at 70%MSO (mean = 0.67, SD = 0.39) and 80%MSO (mean = 0.59, SD = 0.30) were not significantly different from those obtained using a conditioning stimulus of 60%MSO ($p = .15$ and $p = .04$, respectively, Bonferroni corrected). For the D70² coil, none of the comparisons reached significance: 40 vs 50 %MSO ($p = .11$), 50 vs 60%MSO ($p = .19$), 60 vs 70%MSO ($p = .28$), 60 vs 80%MSO ($p = .98$). Using Wilcoxon signed rank tests, no significant differences between intensity levels were found for either model. As hypothesized, these outcomes suggest that CBI may be sufficiently elicited by intensities as low as 60%MSO with a DC coil; however, there is no evidence to suggest that CBI can be elicited at any of the chosen intensities with the D70² coil. Figure 5 shows CBI ratios across intensities for each coil, whereby a progressive reduction with increasing intensity is apparent for the DC coil, but not the D70².

DC Coil Gap and Correlation of CBI with RMT

A noticeable gap between the DC coil and participant scalp was observed in eight of the 13 participants. Correlation analyses (Kendall’s tau) between CBI ratios at each intensity and presence of coil gap showed no significant relationships (see Table 4).

Similarly, there was no significant correlations between CBI ratios at each intensity and participant RMT (Table 5).

VAS

Descriptive statistics for the VAS are shown in Table 6. The outcomes of Wilcoxon signed rank tests are shown in Table 7. Here, comparisons across coils for each intensity showed that the DC coil was significantly less tolerable than

Table 3 Wilcoxon signed rank comparisons for stimulation conditions at each intensity as %MSO

Condition (Stim. Cond-%MSO)	DC coil		D70 ² coil	
	<i>p</i>	Effect size (<i>r</i>)	<i>p</i>	Effect size (<i>r</i>)
TS-40 vs CSTS-40	.84	0.06	.03	0.61
TS-50 vs CSTS-50	.01	0.69	.68	0.11
TS-60 vs CSTS-60	.003	0.81	.50	0.19
TS-70 vs CSTS-70	.003	0.83	.73	0.10
TS-80 vs CSTS-80	.008	0.74	.09	0.47

Stimulation condition: *TS* test stimulus alone, *CSTS* conditioning stimulus preceding test stimulus

the D70² over all scales and intensities (Bonferroni corrected at $p = .05$ for five comparisons) (see Fig. 6 also). When comparisons were made across intensities within a coil (Bonferroni corrected for five comparisons within coil), the DC coil was significantly more painful (scale 1) as intensity increased, excluding the jump from 60 to 80%MSO, which showed a trend toward significance ($p = .01$). This suggests that pain perception may reach a ceiling at higher intensities with the DC coil. Conversely, pain perception for the D70² coil did not increase significantly with increasing intensity. Regarding muscle activation (scale 2), the jump from 50 to 60%MSO only showed a significant increase in muscle activity for the DC coil. Conversely, for the D70² coil, muscle activation was significantly apparent at intensity increments from 60 to 70%MSO and 60 to 80%MSO. These outcomes may suggest that because muscle activation with the DC coil is clearly apparent at lower intensities, a ceiling may be reached at mid-range intensities. Conversely, for the D70² coil, intensities over 60%MSO are required to produce noticeable effects. When assessing general discomfort (scale 3), the DC coil showed a consistent increase in discomfort as intensity increased. A similar outcome was observed for the D70² coil, although only a trend toward significance

occurred for the increment from 50 to 60%MSO ($p = .07$). As this intensity level is within middle range of those tested, the perception of discomfort experienced going from 50 to 60%MSO may have been subjectively less than the initial jump from 40 to 50%MSO due to a lack of reference sensation at these early intensities. Then, discomfort subsequently increased at intensities over 60%MSO.

Discussion

Cerebellar-M1 connectivity has been found to be instrumental in several aspects of motor-function, such as motor learning and adaptation. This connectivity has been successfully assessed in vivo via a dual-coil TMS protocol. However, the DC coil typically used to deliver the cerebellar pulse is reportedly uncomfortable, leading to participant drop-outs and reduced trial numbers in some instances [26, 35, 36]. While flatter coils appear to increase tolerability of the stimulus, they have been shown to be unreliable in the elicitation of CBI, likely due to insufficient depth range [13, 37]. The current feasibility study investigated the range of intensities over which CBI is reliably observed when using both the

Fig. 5 Mean CBI ratios across intensities for double cone (DC) and D70² (F8) coils. A progressive reduction with increasing intensity is apparent for the DC coil, but not the D70². Error bars represent ± 1 SEM

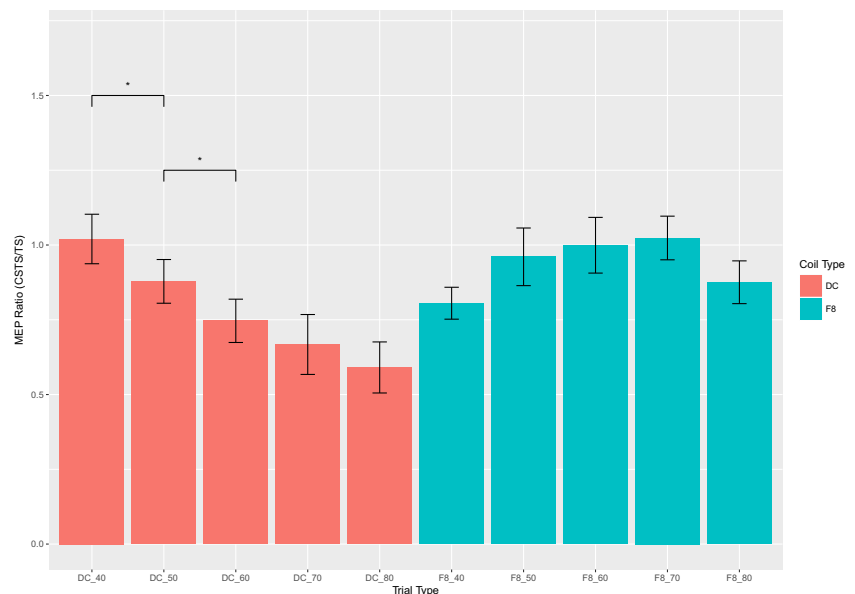


Table 4 Correlation analyses (Kendall's tau) between CBI ratios at each intensity and presence of gap between participant scalp and convergent region of DC coil showed no significant relationships

Intensity (%MSO)	T	P
40	0.25	.30
50	−0.25	.30
60	−0.25	.30
70	0.26	.34
80	−0.05	.85

traditional DC coil and a newer, higher-powered figure-of-8 coil, the D70². Tolerability of each intensity was also assessed.

As hypothesized, it was found that CBI was reliably elicited at CS intensities upwards of and including 60%MSO with the DC coil. Furthermore, the degree of suppression elicited at 70%MSO and 80%MSO with this coil was not significantly different to that at 60%MSO, suggesting that stimulating at intensities above 60%MSO may be unnecessary in the majority of cases. Conversely, conditioning stimuli delivered with the D70² coil did not reliably elicit CBI at any of the five intensities tested. Tolerability measures for each coil confirmed the hypothesized relationship between coil type and participant discomfort, with the DC coil being significantly less tolerable than the D70² coil over all intensities on measures of pain, muscle activation, and general discomfort. Furthermore, perceived pain and general discomfort tended to increase with increasing intensity when stimuli were delivered with the DC coil.

These findings suggest that, while the DC coil is less tolerable than the D70², the additional depth range achieved by the angled design is necessary to provoke activation of PCs in the cerebellar cortex, leading to CBI. The results of this study are in line with findings from Hardwick, Lesage [13] and Werhahn, Taylor [37], where consistent suppression following DC stimulation, but not with a flat figure-of-eight coil was reported. Specifically, Hardwick, Lesage [13] reported that CBI was

Table 5 Correlation analyses (Kendall's tau) between CBI ratios at each intensity participant RMT showed no significant relationships for either coil

Coil	Intensity (%MSO)	T	p
DC	40	0.19	.61
	50	0.26	.47
	60	−0.07	.86
	70	0.07	.88
	80	0.10	.84
D70 ²	40	0.08	.84
	50	0.01	1.00
	60	0.66	.16
	70	−0.32	.37
	80	−0.54	.11

Table 6 Descriptive statistics for VAS scale score [1–10] at each stimulator intensity (%MSO) for each coil

Intensity (%MSO)/ VAS scale	Mean	Median	SD	SEM	
DC coil					
40	1	1.35	0.90	1.59	0.44
	2	3.38	3.20	2.26	0.63
	3	2.12	1.60	1.71	0.48
50	1	2.22	2.20	1.90	0.53
	2	4.35	4.10	2.39	0.66
	3	3.55	3.40	1.81	0.50
60	1	3.62	3.30	2.64	0.23
	2	5.38	5.40	2.56	0.71
	3	4.83	4.70	2.37	0.66
70	1	3.72	4.10	2.87	0.87
	2	5.61	5.70	2.20	0.66
	3	5.43	5.40	2.33	0.70
80	1	5.01	2.00	1.96	0.54
	2	6.86	7.50	3.87	0.75
	3	6.85	6.95	1.94	0.62
D70 ² coil					
40	1	0.25	0.10	0.29	0.08
	2	0.55	0.30	0.61	0.17
	3	0.37	0.30	0.44	0.12
50	1	0.65	0.30	0.83	0.23
	2	1.18	0.70	1.34	0.37
	3	0.81	0.40	1.04	0.29
60	1	1.12	0.50	1.48	0.41
	2	1.66	1.30	1.70	0.47
	3	1.02	0.50	1.17	0.32
70	1	1.55	1.30	1.75	0.49
	2	2.29	1.50	2.08	0.58
	3	1.60	0.70	1.59	0.44
80	1	2.09	2.00	1.95	0.54
	2	3.12	2.40	2.48	0.69
	3	1.98	2.40	1.67	0.46

Scale 1: level of pain experienced from cerebellar stimulation; scale 2: degree of muscle activation experienced during cerebellar stimulation; scale 3: perceived level of general discomfort from cerebellar stimulation

reliably elicited via a 110-mm DC coil at intensities of 65, 70, 75, and 80%MSO, whereas a wider-angled 'batwing' coil elicited CBI only at higher intensities (75 and 80%MSO). A 70-mm figure-of-8 coil was found to be inconsistent across all intensities. The current study extends these findings to include suppression with a DC coil at 5% below the previously tested range. Furthermore, it confirms that the increased focality of the D70² coil over earlier figure-of-8 models does not improve the reliability with which CBI may be elicited when used for the CS.

In light of these findings, a pragmatic approach to CBI assessment may be to stimulate each participant at

Table 7 Wilcoxon signed rank comparisons across VAS scales for stimulation conditions at each intensity as %MSO

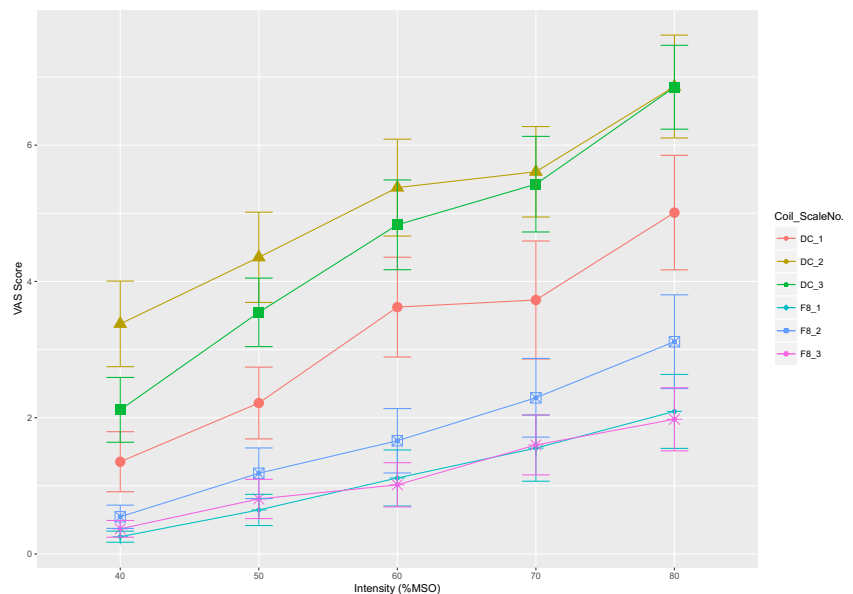
Condition (Stim.cond-%MSO)	VAS scale 1		VAS scale 2		VAS scale 3	
	<i>p</i>	Effect size (<i>r</i>)	<i>p</i>	Effect size (<i>r</i>)	<i>p</i>	Effect Size (<i>r</i>)
Coil-coil comparisons:						
DC-40 vs D70 ² -40	.004	−0.79	.002	−0.85	.002	−0.85
DC-50 vs D70 ² -50	.005	−0.78	<.001	−1.02	<.001	−0.98
DC-60 vs D70 ² -60	.003	−0.82	<.001	−1.02	.001	−0.88
DC-70 vs D70 ² -70	.005	−0.78	.003	−0.81	.004	−0.79
DC-80 vs D70 ² -80	.002	−0.86	.002	−0.86	.002	−0.86
Intensity-intensity comparisons:						
DC-40 vs DC-50	.003	−0.81	.01	−0.70	.001	−0.88
DC-50 vs DC-60	.002	−0.84	.004	−0.79	.002	−0.84
DC-60 vs DC-70	.002	−0.84	.02	−0.64	.008	−0.74
DC-60 vs DC-80	.01	−0.69	.02	−0.67	.002	−0.86
D70 ² -40 vs D70 ² -50	.01	−0.68	.05	−0.54	.007	−0.75
D70 ² -50 vs D70 ² -60	.09	−0.47	.02	−0.63	.07	−0.51
D70 ² -60 vs D70 ² -70	.02	−0.64	.001	−0.88	.002	−0.86
D70 ² -60 vs D70 ² -80	.01	−0.72	.006	−0.76	.008	−0.74

Stimulation condition: *TS* test stimulus alone, *CSTS* conditioning stimulus preceding test stimulus

60%MSO with the DC coil while assessing for spinal artefacts off-line. However, there are limitations to this recommendation. Firstly, earlier studies have found that the degree of suppression following the CS increases with increasing intensity [11, 29]. Hence, given individual differences in variables which affect neural response to TMS, such as scalp-to-neural-site distance [44], CBI may not be elicited at low-to-mid intensities in some participants. To cater for these cases, on-line inspection of MEP amplitude comparing responses both after the TS alone and after conditioning may be sufficient to determine if higher intensities are necessary. Secondly, using a fixed intensity for the CS rather than one specific to an individual may increase trial variability, with some participants showing lower and others higher levels of CBI at the chosen intensity. However, ensuring study design is within-

subjects will account for some of this variation, which may be appreciably less than the variability introduced by stimulus intolerance. Thirdly, using a fixed intensity does not necessarily control for spinal artefacts, and off-line assessment may result in a loss of data. While we found less than 1% of trials contained evidence of spinal artefacts over the tested range, antidromic collaterals are a significant cause for concern when applying TMS to cerebellar regions [21]. Thus, consistent with recommendations by Ugawa, Uesaka [11] and Fisher, Lai [21], an assessment of the direct threshold necessary to elicit activation of the corticospinal tract should be taken to control for spinal influences contributing to any observed suppression of MEPs, and intensity determined relative to this. In light of findings from the current study, stimulation intensity could then be capped in the range of 60%MSO providing

Fig. 6 Mean VAS score grouped by coil type, double cone (DC) and D70² (F8), and scale number, 1: pain scale, 2: muscle activation scale, 3: general discomfort scale. The DC coil scored significantly higher than the D70² on all measures over all intensities (%MSO). Error bars represent ± 1 SEM



direct threshold does not fall below this. However, as direct motor threshold cannot be elicited in all individuals [14], a fixed intensity in the order of 60%MSO is a reasonable default. Furthermore, the use of a low and narrowed intensity range in conjunction with off-line assessment of MEP traces for spinal artefacts may be used in some vulnerable populations, such as clinical groups (e.g., autism spectrum disorder), and the elderly [12]. Although, when no CBI is evoked in patient groups with cerebellar dysfunction via a fixed, low intensity, a higher CS should alternatively be used. In such cases, CS intensity may be determined via the threshold of corticospinal tract activation at the brainstem [11, 22].

Several CBI studies have determined CS intensity based on participant RMT [35, 38, 45, 46]. In the current study, no correlations between RMT and individual's degree of suppression across intensities were found. This suggests that cortical measures such as RMT are not likely representative of thresholds required to elicit CBI. This is not surprising, due to the vast differences between cortical motor and cerebellar/spinal pathways. Hence, CS intensity should not be determined as a proportion of cortical motor thresholds. Instead, individualized intensity may be defined as a proportion of AMT, elicited via a direct stimulus given at the level of the foramen magnum [11, 22], or via offline inspection of MEP latencies [12, 13, 21].

Furthermore, no relationship between the coil-to-scalp gap in the DC coil and suppression was observed. While this is encouraging, to obtain a true measure of gap effects, an equivalent stimulus flush with the scalp would also need to be delivered. Determining how to deliver this 'equivalent stimulus,' however, would be non-trivial and further investigation into resolving this issue is warranted.

It must also be stressed that these recommendations are only valid for the stimulus locations and ISI presented in this paper. It is possible that other intensities may be more appropriate for different parameter values. For instance, CBI may be elicited at ISIs between 5 and 8 ms, and from different locations at the back of the head [11]. However, it is not expected that varying the ISI will greatly change the intensities at which CBI is elicited, although some small differences may occur when varying stimulation site due to variation in scalp-to-cerebellar-site distance [13]. Furthermore, the parameter values chosen in this study are among the most commonly used in the CBI literature [14]; hence, the findings presented in this paper are applicable to the majority of CBI studies for healthy adult participants. However, it is possible that other ISIs may be more appropriate for different coil designs. This should be investigated in the future. It is also acknowledged that a threshold difference may occur between different racial groups (for instance, Asian versus Caucasian). While the sample in this study did test over a variety of racial groups, Caucasians made up the racial majority, and race was not controlled for in the final analysis. Thus, it is possible that the outcomes presented are most applicable for those participants of Caucasian background.

Conclusion

Tolerability to stimuli is rarely reported in TMS literature [47]; however, intolerance has the potential to lower the power, reliability, and validity of a study due to participant drop-outs, incomplete trials, and muscle artefacts. Muscle tension may be of particular concern when TMS is incorporated with highly sensitive measures of neurophysiological activity such as EEG, where muscle activation (particularly jaw-clenching) may flood measurement channels [48]. The current study found that a cerebellar stimulus of 60%MSO with a DC coil was sufficient to elicit CBI in the majority of participants, whereas the D70² did not reliably elicit CBI over all intensities tested. Thus, while the DC coil was found to be significantly less tolerable than the D70², the superior depth-characteristics of the angled coil are necessary to provide reliable elicitation of CBI. A mid-range intensity such as 60%MSO may provide a practical compromise between study validity and participant well-being, particularly in vulnerable populations.

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Compliance with Ethical Standards

Ethical approval was granted by the Deakin University Human Research Ethics Committee.

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

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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