A Novel Protocol to Investigate Motor Training-Induced Plasticity and Sensorimotor Integration in the Cerebellum and Motor Cortex

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Abstract

Our study set out to develop a sensitive technique, capable of detecting output changes from the posterior fossa following a motor acquisition task. Transcranial magnetic stimulation (TMS) was applied over the right cerebellar cortex 5 ms in advance of test stimuli over the left cerebral motor cortex (M1), suppressing test motor-evoked potentials (MEPs) recorded in a distal hand muscle. Ten participants typed the letters Z,D,F,P in randomized eight letter sequences for approximately 15 minutes, and ten participants participated in the control condition. Cerebellar-M1 recruitment curves were established prior to and after the motor acquisition task. Cerebellar inhibition at 50 percent (CBI50) was defined as the intensity of cerebellar-M1 stimulations that produced MEPs that were 50 percent of the initial test MEP. Collection also occurred at stimulator intensities 5% and 10% above CBI50. A significant interaction effect of group (experimental and control) versus time (pre- and post-intervention) was observed \[ F(1,18) = 4.617, p = 0.046 \]. Post-hoc tests showed a significant effect for the learning task in the experimental group \[ F(1,9) = 10.28, p = 0.01 \]. Further analysis showed specific dis-inhibition at CBI50 \( p = 0.04 \), CBI50+5\% \( p = 0.008 \), and CBI50+10\% \( p = 0.01 \) for the experimental group only. Reaction time \( p < 0.001 \) and accuracy \( p = 0.006 \) improved significantly following practice, implying that dis-inhibition coincides with motor learning. No changes however were seen in the control condition. We conclude that this protocol is a sensitive technique that may be used to study cerebellar dis-inhibition with motor acquisition \textit{in vivo}.

Keywords: cerebellum, non-invasive cerebellar stimulation, transcranial magnetic stimulation, motor acquisition task, plasticity, sensorimotor integration
Introduction

Transcranial magnetic stimulation (TMS) has been used extensively to study changes in neural plasticity coupled with behavioural learning. Most TMS studies have specified anatomical regions within the cerebral cortices, and more specifically within the primary motor cortex (M1), when considering changes that occur with motor learning (Classen et al. 1998; Liepert et al. 1998; Pascual-Leone et al. 1995; Sugawara et al. 2013). There are however few studies that involve the cerebellum and associated components of the posterior fossa and lower brain stem.

Ugawa et al. (1995) was among the first to pilot magnetic stimulation over the cerebellum through the use of a double-cone coil that could stimulate deep, underlying tissues. To obtain the desired response, Ugawa et al. (1995) applied a conditioning stimulus to the posterior fossa 5 to 8 milliseconds (ms) prior to a test stimulus over the contralateral M1, exciting cerebellar neurons prior to the motor command volley. Ugawa et al. (1995)’s original design relied on “finding” the threshold for eliciting a cervicomedullary evoked potential (CMEP); once this CMEP threshold was found, the desired intensity was determined using a stimulation intensity 5 to 10% below CMEP threshold to stimulate the cerebellum, based on the rationale that this would minimize contamination of the trace with CMEPs (Ugawa et al. 1995).

This feature of their protocol design poses a difficulty: CMEPs are of a low magnitude, they can be painful to participants due to the high intensities of stimulator output and the large concomitant contraction of the dorsal neck muscles, and additionally, they are not able to be elicited in all subjects (Martin et al. 2009). The main strength of Ugawa et al. (1995)’s original method was that it ensured no CMEP activity during cerebellar stimulations. CMEPs however occur at varying intensities of stimulator output dependent upon individual subjective variables, such as size of subject’s head, density of neurons, skull thickness, and inherent excitability of...
neurons in the activated pathway (Martin et al. 2009). The intensity Ugawa et al. (1995) used to
elicit CMEPs and the location over the inion, in contrast to the more lateral placement for
cerebellar stimulations, does not provide a physiological baseline that may then be used to assess
cerebellar activity in experimental designs. An ideal method should produce test MEP inhibition
of approximately 50 percent to allow for group comparisons of cerebellar inhibition following
experimental interventions and to prevent the possibility of floor or ceiling effects when used for
individual subject analysis.

A non-invasive approach such as magnetic stimulation over the cerebellum is a
favourable method to investigate changes in cerebellar output that occur with motor acquisition,
as the cerebellum is a structure known to be \textit{a priori} implicated in the general processes of
sensorimotor integration and motor learning (Doyon et al. 2003; Doyon et al. 2002; Manzoni
2007; Penhume and Doyon 2002). Therefore, experimental designs are needed to accurately
study human cerebellum function \textit{in-vivo}. Studies that do exist on the sensorimotor role of the
cerebellum are often performed using advanced imaging modalities such as MRI, which places
subjects within a restricted area, generally in a supine position and with limited dynamic task
interactive capability. A unique advantage of TMS is that it may be applied with relative ease
within a dynamic paradigm when used to assess structural or functional changes following task
acquisition.

Our study set out to extend and refine Ugawa et al. (1995)’s previous work, evaluating
potential cerebellar effects while minimizing contamination of the test MEP with CMEPs and
cervical root evoked potentials. We hypothesized that following the learning of a motor
acquisition task similar to tasks shown through MRI studies to activate cerebellar nuclei and
regions of the cerebellar cortex (Doyon et al. 2002; Penhume and Doyon 2002), we would see
dis-inhibition of cerebellar output as an outcome of sensorimotor changes within the cerebellum.

Methods

Subjects

Twelve healthy volunteers (6 females) were tested to see whether stimulations over the
cerebellum would produce extraneous activity within the electromyographic (EMG) trace. All
subjects showed inhibition of the test MEP with cerebellar-M1 stimulations (ISI 5 ms). However,
in one subject, cervical root activity was apparent, specifically EMG activity with a latency of
approximately 14 ms from the stimulus. In another subject, motor activity was identified due to
latency changes within the EMG trace. EMG activity with a latency approximately 5 ms in
advance of the test MEP was considered to be motor cortex activity, produced by the firing of the
double-cone coil (which was fired 5 ms in advance of the test MEP stimulus), and was thus not
created by the cerebellar-M1 conditioning. This extraneous activity potentially depicts
antidromic activation of the pyramidal tract (Ugawa 2009; Ugawa et al. 1991). Due to potential
confounding effects, traces with this activity were not included in the analysis. Data from these
two subjects were excluded from the study so that out of the twelve original subjects, ten were
included in the final analysis (4 females, mean age 22.7 years, range 19 to 27 years). Seven of
these subjects were right-handed and the other three were ambidextrous. Thirteen control
subjects (6 females) were also tested, and of these control subjects, two showed motor cortex
activity and one showed cervical root activity. A total of ten were included in the final analysis
(4 females, mean age 22.6 years, range 20 to 27 years). Nine of these controls were right-handed
and one was ambidextrous.
Inclusion and exclusion criteria

Inclusion criteria for the study included age between 18 and 45 years. Participants were excluded if they had any contraindications to TMS such as history of epilepsy, presence of metal fragments in the upper body or eye, subject taking neuroactive medications, heart disease, skull fracture or serious head injury, and pregnancy. Each subject gave their written and verbal consent. The study received ethical approval from the university’s Research Ethics Board (University of Ontario Institute of Technology, Oshawa, Ontario) in accord with the Declaration of Helsinki statutes governing research on human subjects.

Experimental setup

Subjects sat upright on a chair with EMG recording electrodes placed on the belly and distal tendon of their tonally quiescent first dorsal interosseus (FDI) of the right hand. The response was recorded as a raw trace that had been amplified (1000x) and band-pass filtered between 20 and 1000 Hz through a CED 1902 interface (Cambridge Electronic Design, Cambridge, England). The signal was sampled at 5 kHz with CED 1401 before recorded as a digital signal in CED Signal 4.08. The figure-of-eight coil (9 cm diameter) connected to a Magstim 200² (Magstim Co., Whitland, Dyfed, UK) and the double-cone coil (11 cm diameter) connected to a Magstim Bistim. The Magstim Bistim allows output from two Magstim 200² units to be channelled into a single coil and enables the output to be 13% greater than the output of a single Magstim 200² (Magstim 2002). Across conditions, stimulator output over M1 was maintained at a constant percent of maximum stimulator output (MSO) ±2% to maintain the test MEP at approximately 0.5 mV in peak-to-peak amplitude.

The double-cone coil was positioned over the cerebellum at the midline between the inion and the external auditory meatus at the level of, or slightly above the level of, the inion to
elicit optimal MEP suppression as described by Ugawa et al. (1995). The coil was held by a Magstim aluminum stand, and cloth tape held the coil so that it was strapped in place to the back of the head in a comfortable, snug placement. A researcher with at least eight months of experience held the figure-of-eight coil over the motor cortex with the handle in a posterior direction at approximately 45 degrees from the sagittal plane. The optimal stimulation site to elicit MEPs from the FDI was marked with a permanent marker onto a tightly-fitted scalp cap. Stimulator output to the figure-of-eight coil was adjusted in small increments to elicit test motor-evoked potentials (MEPs) of approximately 0.5 mV peak-to-peak amplitude averaged across 16 sweeps. This stimulator intensity was selected since test MEPs under 1 mV have been shown to be most sensitive to effects of inhibition (Daskalakis et al. 2004; Ugawa et al. 1995). Rest threshold (RTh) was found over M1 as the lowest stimulator output that elicits five out of ten significant MEPs with peak-to-peak amplitude equal to or greater than 50 µV. 

Inhibition of the test MEP (Fig. 1A.) was produced through firing the double cone coil over the cerebellum 5 ms in advance of the figure-of-eight coil over the M1. The traces were then carefully examined for instances of extraneous activity (corticospinal activity, cervical root activity, antidromic activity) and these individual traces, along with traces that showed background EMG, were removed so that 8 to 10 MEPs were averaged for each level of stimulus intensity. Examples of this extraneous activity may be seen in Figure 2, which depicts corticospinal activity (Panel A) and cortical root activity (Panel B). The small CMEP size in Figure 2 Panel A shows that careful examination at high gain is necessary to determine the data traces that may be affected. An unusually large MEP occurs at Figure 2 Panel A, and the researchers also noted that when no CMEPs were apparent in this subject, smaller MEPs were
produced. This indicates that CMEPs very likely have an effect on the size of the MEPs, and that visibly analyzing the trace will enable one to determine the traces that are contaminated and to differentiate these from the traces that are free of extraneous activity.

**FIGURE TWO WILL APPEAR APPROXIMATELY HERE**

**Cerebellar stimulations**

To determine cerebellar inhibition at 50 percent (CBI50) we carried out the following protocol. One single pulse was delivered through the double cone coil over the cerebellum, to be used in later analysis as a buffer and also to evaluate the effect of cerebellar stimulations on the EMG trace, and ten cerebellar-M1 stimuli pairs (ISI 5 ms) were delivered at each of six to ten intensities in 5% MSO intervals (e.g. 55, 60, 65, 70, 75, 80, 85% MSO) for a total of eleven stimulations at each cerebellar stimulation intensity level. Higher levels above 90% MSO were not used to decrease the probability of eliciting CMEPs, which would confound the interpretation of cerebellar changes. For each subject, the stimulator was set to an individualized level determined by the subject’s RTh and the distance of the coil to the head. Individualized levels were used due to potential effects on inhibition caused by the size of the subject’s head, skull thickness, gender, RTh and age. Subjects’ data tended to show a downward slope with increasing stimulus intensities (Fig. 3A.). The level where there was approximately 50 percent inhibition, with an increase in inhibition seen in adjacent level(s), was defined as CBI50 (Fig. 3B.). At each cerebellar stimulation intensity, the double-cone coil was discharged over the cerebellum on its own, and the EMG trace was carefully observed for the presence of CMEPs or cortical root activity. Martin et al. (2009) have identified CMEPs detected in the FDI to have a latency of approximately 18 ms and cervical root activity to have a latency of approximately 15 ms, whereas cerebellar-M1 stimulations should elicit MEPs with a latency comparable to each
subject’s test MEP at approximately 21 ms. Only one subject of the ten showed cervical root activity (<3% of the traces) and the individual traces showing this activity were not included in the final analysis.

Data analysis

Once the traces for inclusion had been visually determined, a buffer was applied to remove the cerebellar stimulus artefact which caused the EMG trace to sweep upward. The buffer was obtained from a trace with a stimulus artefact of similar amplitude to the cerebellar-M1 stimulation but without a MEP, and this buffer was subtracted from each stimulation prior to averaging (Wichmann 2000). The buffered data was rectified and the response was determined as the area under the curve of the rectified averaged EMG (conditioned response) over the area of the test MEP (unconditioned response). In a separate analysis, peak-to-peak amplitude of the unbuffered data was determined, in order to assess the feasibility of this method, as compared to area under the rectified averaged curve.

Cerebellar inhibition at 50 percent

Stimulations were collected at incrementally increasing levels of conditioning stimulus intensity. The stimulation intensity that produced a value that was the closest to 50 percent inhibition, followed by a similarly inhibited MEP at the next stimulation increment, was determined for each individual subject to enable comparisons between subjects (Fig. 3). We made comparisons at CBI50, CBI50+5% stimulator output and CBI50+10% stimulator output. The levels CBI50, CBI50+5%, and CBI50+10% were the only levels selected for statistical analysis, as some subjects started to inhibit at 50 percent as soon as they started the protocol and never received stimulator intensity less than CBI50. Other subjects inhibited only at 80% MSO
and, as we intentionally did not collect levels above 90% MSO, we only reached CBI50+10% in these participants.

Motor acquisition task

In the experimental group, participants typed randomized eight-letter sequences of the letters Z, P, D, and F with the right index finger (Z,D,P,Z,F,P,D,D). This typing task, programmed in E-Prime 2.0 software (Psychology Software Tools, Sharpsburg, Pennsylvania), took approximately 15 minutes to complete and was selected as similar tasks have been shown to activate the cerebellum in early stages of motor sequence learning (Doyon et al. 2002; Penhume and Doyon 2002). The movement is also known to replicate finger-tapping which has been demonstrated through functional magnetic resonance imaging and positron emission tomography to activate the cerebellum (Olsson et al. 2008; Stoodley et al. 2012; Witt et al. 2008). Electrodes placed on the FDI recorded background muscle activity, and subjects completed ten randomized sequences of the eight letters at the start and end of the motor acquisition task to evaluate accuracy and reaction time.

Control condition

The control intervention was carried out to ensure that any changes observed following the motor acquisition task were specific to the motor task and not due to potential methodological confounding variables involved with re-testing of the protocol a second time (e.g. removal and replacement of the stimulating coils). Instead of engaging with the task, subjects sat on a chair with their FDI muscle quiescent. The investigators removed the TMS coils (as was done for the motor task in the experimental group) and replaced the TMS coils shortly thereafter. Identical TMS coil placement is vital for reproducibility, so this was attended to very carefully for both groups by the data collection team.
Statistical analysis

The p-value was set to 0.05, and statistical analysis was performed through IBM SPSS Statistics 19. Repeated-measures, mixed-design analysis of variance (ANOVA) assessed interaction between inhibition levels before and after the intervention (two levels: pre-intervention and post-intervention), across stimulator intensities (three levels: CBI50, 5% MSO above CBI50 and 10% MSO above CBI50), and across groups (two groups: experimental and control). Post-hoc repeated-measures ANOVAs and paired-sample t-tests compared the levels where significance was seen in the mixed-design ANOVA. Mean reaction time was found for each subject, and the differences between the group means were assessed with the paired-sample t test. Accuracy was tallied for each subject out of a maximum accuracy response of 80 (a score of 80 = 100% accuracy). The sum of group accuracy was tallied and assessed with the chi-squared test comparing changes to accuracy before and after the practice.

Results

Coupling the cerebellar and M1 stimulations (ISI 5 ms) caused MEPs to have a decreased size. This phenomenon can be seen in Figure 1. Figure 1 also shows that following the motor learning task, the cerebellar-M1 stimulations (ISI 5 ms) produced MEPs of a larger size than the MEPs produced during the pre-motor learning condition. A general trend was noted where inhibition increased (e.g. smaller MEP size) at increasing stimulus intensities for individual subjects (Fig 3A.). However, the stimulus intensity at which CBI50 occurred varied between individual subjects, as depicted in sample traces for three individual subjects (Fig. 3A). Once CBI50 had been determined as the response that is fifty percent inhibition with adjacent responses showing similar inhibition, the corresponding CBI50 curves were plotted, as depicted
for the same three subjects (Fig. 3B.). Figure 3 shows that plotting the CBI50 curves in this way standardizes a physiological inhibition level and enables group comparisons.

A significant interaction effect of group (experimental and control) versus time (pre-intervention and post-intervention) was observed \[F(1,18) = 4.617, p = 0.046\]. Post-hoc tests showed a significant effect following the learning task in the experimental group \[F(1,9) = 10.28, p = 0.01\], whereas no significant effect was seen after the control condition. Post hoc two-tailed paired \(t\) tests showed significance for the experimental group at CBI50 \((p = 0.04)\), CBI50+5% \((p = 0.008)\), and CBI50+10% \((p = 0.01)\) (Fig. 4). The average MEP size following the motor acquisition task increased from 52.7% to 112% at CBI50; from 38.9% to 101% at CBI50+5% and from 41.2% to 90.1% at CBI50+10% (Fig. 4A.). In the control condition, the change in average MEP size was much less with changes from 50.6% to 60.6% at CBI50; from 41.3% to 56.9% at CBI50+5% and from 40.9% to 52.8% at CBI50+10% (Fig. 4B.).

In a separate analysis, peak-to-peak amplitude, without application of a buffer, was used to assess similarities between analysis methods. When the same intensities used in group comparisons above were applied in the experimental group only, the repeated-measures ANOVA test showed a similar difference in pre- versus post-intervention measurements \([F(1,9) = 11.13, p = 0.009]\) with post hoc analysis showing significance at CBI50 \((p = 0.03)\), CBI50+5% \((p = 0.004)\), and CBI50+10% \((p = 0.01)\). No apparent differences were seen between the two analysis methods.
Motor acquisition task

For the experimental group, two-tailed paired sample \( t \) tests showed significant improvement to reaction time following the 15 minute task \((p < 0.001)\). As well, the chi-squared test showed that accuracy improved significantly following the task \((p = 0.006)\) (Fig. 5.).

Discussion

This study presents a novel method to non-invasively explore cerebellar dis-inhibition of M1 in vivo in human subjects. This study demonstrated that, following a motor acquisition task, there was reduced inhibition following pairs of cerebellar-M1 stimulations (ISI 5 ms). This was seen at three different intensities: at the level where approximately 50 percent of the test stimulus over M1 was observed prior to the intervention (what we have termed cerebellar inhibition at 50 percent, or CBI50); at 5% MSO above this (termed CBI50+5%) and at 10% MSO above this level (termed CBI50+10%), suggesting there are physiological changes that occur following a motor acquisition task which involve cerebellar-M1 pathways. It is advantageous to compare the data at least at three stimulation intensities to provide a more robust measure of the degree of inhibition, and this is recommended for this type of study due to fluctuations that may occur when inhibition is investigated at a single stimulation intensity.

Novel method

A similar study on cerebellar changes (Daligadu et al. 2013) used absolute values of conditioning stimulus output (as opposed to values relative to CBI50) following a motor task with the numbers 7, 8, 9 repeated in randomized six number sequences (7,9,8,8,7,7). Stimulator intensities of 70, 80, and 90% MSO were applied to the posterior fossa with a constantly-maintained stimulator output over M1. The results of the Daligadu et al. (2013) study showed no
change in inhibition ratios at each of the above stimulator intensities as determined in healthy subjects. However, in the current study, when comparisons were made with selected stimulator intensities to elicit 50-percent EMG-MEP suppression and individualize the stimulator intensity to CBI50, clear changes were evident as seen in the aggregated group data (see Fig. 4).

*Potential mechanisms for dis-inhibition*

It is generally accepted that a principal function of the cerebellum is to modify or refine extracerebellar output through the mechanism of inhibition sourced from GABAergic neuron populations. Various imaging studies report that the cerebellum is active during motor sequence tasks (Doyon et al. 2002) and finger-tapping tasks (Olsson et al. 2008; Stoodley et al. 2012; Witt et al. 2008). It also participates in, and plays an active role in, motor adaption and in the behavioural learning of unfamiliar tasks (Doyon et al. 2003).

In this study the two stimulated areas were the cerebellum and primary motor cortex (M1). The test MEPs elicited over M1 were matched between and across subjects prior to and following the motor acquisition task at a value close to 0.5 mV. Stimulator output over M1 was maintained at a constant percent MSO ±2% across conditions. Changes were seen at CBI50 following the motor acquisition task but not seen in the control, providing support to the proposition that the motor acquisition task induces changes within or between the cerebellum and M1 (the two stimulated areas) and that these changes are able to be detected by the cerebellar-M1 protocol (ISI 5 ms) described in this study.

We speculate that there are two intrinsic mechanisms that may contribute to dis-inhibition at CBI50 as seen in this study. The first possible mechanism is that during stimulation over the posterior fossa, there may be an increased discharge from collaterals of both mossy and climbing fibres that synapse onto cerebellar nuclei. This proposition is supported by the principle that
sensory neurons synapse onto cerebellar nuclei before synapsing with deeper Purkinje cells within the corpus of the cerebellum, and prior work supports that these sensory neurons produce a resultant increased discharge of cerebellar nuclei with voluntary movement (Holdefer et al. 2005).

A second possible mechanism contributing to dis-inhibition following the motor acquisition task is the activity from sensory neuron complexes that project to the cerebellum as a result of the motor task. This proposition is supported by previous animal studies which have shown encoding of limb loci in the spinocerebellar tract (Bosco et al. 1996) and also in the external cuneate nucleus projecting to both the thalamus and cerebellum (Giaquinta et al. 1999). Previous work has also shown that practice of a motor sequence acquisition task, similar to the one used in this study, causes near-to-constant activity within the cerebellar cortex during early and late stage phases of learning and adaption (Doyon et al. 2002). Interestingly, despite this near-to-constant observed activity of the cerebellar cortex, “increased activity” within the associated nuclei could be seen following thirty minutes of practice (Doyon et al. 2002). This increased cerebellar nuclei activity following practice of the task is indicative that changes occur upstream of Purkinje cells (e.g. within sensory fibres). These findings support our hypothesis that encoding in sensory neurons, and possibly long term depression (LTD) of parallel fibre connections described by Ito (2006), play a role in changes to the output produced at cerebellar nuclei and in the dis-inhibition at CBI50 as seen in this study.

Motor cortical hyperexcitability following the task, however, is a mechanism that does not fully explain the change seen after the motor acquisition task. Hyperexcitability would mean that the motor cortical test MEP (produced from 16 sweeps) would show an increase in size (Delvendahl et al. 2012). However, each MEP produced by the cerebellar-M1 stimulations was
normalized, using the motor cortical test MEP in the denominator and the cerebellar-M1 MEP in the numerator. Based on this normalization process, large amounts of motor cortical hyperexcitability (and larger motor cortical MEPs) would cause there to be a much smaller “conditioned MEP response.” Similarly, if there were to be much less excitability of the motor cortex, this would cause there to be a larger “conditioned MEP response.” Normalization means that small changes in excitability after the motor acquisition task would not have affected the outcome. In the study, a much larger “conditioned MEP response” was seen after the intervention even though the motor cortical test MEP was the same as prior to the motor training (e.g. the same size at the same percent MSO ± 2%). This suggests that the main source of the inhibition was due to changes in excitability in the cerebellar-M1 pathway, as distinct from changes in excitability within M1.

Motor-training plasticity

In this study, reaction time improved significantly following the 15 minute motor acquisition task. Accuracy also improved significantly following the task, coinciding with disinhibition at CBI50. Given the large sample size of 800 responses from the motor acquisition task, it is unlikely that these changes to accuracy happened by chance alone. There is strong support that the cerebellum is highly active in motor adaptation and learning, especially in early stages (Doyon et al. 2003; Doyon et al. 2002), and the combined effect of improved reaction time and accuracy following practice suggest both plasticity and a learned effect subsequent to motor skill acquisition; as well, it is highly likely that multiple changes occur along the cerebellar-M1 pathway subsequent to this acquisition task.
Sensorimotor integration related to task acquisition

Motor skill acquisition requires processing somatosensory information received from the motor task and integrating this information with the motor command (or efference copy) sent from M1, to fine tune and improve the efficacy of the motor task performed. This is referred to as sensorimotor integration. The cerebellum is known to be highly involved in sensorimotor integration, both receiving information from the motor cortex regarding motor commands and integrating this with peripheral sensory information and expected sensory information due to past experience (Manzoni 2007; Nixon 2003), to enable fine tuning and improved efficacy in task performance. In this study, stimulation of the cerebellum was performed 5 ms prior to stimulation of M1 creating inhibition within a distal hand muscle. The literature shows that intervals less than 5 ms or greater than 8 ms resulted in no change to cerebellar output (Ugawa et al. 1995), and it appears that 5 to 8 ms is optimal for the merging of the two signals from the posterior fossa and M1. This time window (5 ms) provides insufficient time for sensory input from proprioception and cutaneous touch to travel to the cerebellum and modify the output. In this study, changes were seen in cerebellar-motor output following the learning of a motor task, implying that the task was encoded as part of the body schema since the time window (5 ms) excludes the possibility of contaminant effects from proprioceptive and cutaneous input, which is a strength of the CBI50 method.

Limitations

A potential limitation of this study is that the control group did not engage in a mentally-stimulating task, whereas the experimental group used both motor and cognitive resources. Although the researchers determined that the effects seen in the experimental group were not
seen within the control, it may be that a task could be used for the control condition that requires mental concentration, but not the learning of movements.

**Potential research for future**

Future applications of this technique could include the study of changes in cerebellar inhibition during different stages of motor acquisition. For example, if greatest dis-inhibition is observed following the initial stages of learning and less dis-inhibition is seen following lengthy periods of practice, these findings would consolidate and extend previous work, possibly formalising the timing paradigm indicating when cerebellar nuclei activity is greatest (Doyon et al. 2002). In later stages of learning, previous work has shown activity to transition from cerebellar nuclei to higher brain regions, specifically the corpus striatum and projections to related motor areas, basal ganglia circuitry and other subcortical structures (Doyon et al. 2003; Doyon et al. 2002; Penhume and Doyon 2002). These studies however used fMRI and PET which depend on vascular perfusion characteristics and changes in blood flow to infer changes in neural activity and require measurements over several minutes, being potentially less sensitive to the immediate changes in inhibition projected from cerebellar neurons to M1. In contrast, the TMS-CBI50 method may be more sensitive to temporal changes, since it may be used immediately following a task or intervention. It is also more specific in targeting neuronal targets and pathways associated with M1 due to localisation characteristics and more specific placement of coils, whereas fMRI and PET reflect the composite neural activity of reciprocal projections throughout the brain and spinal cord (Manzoni 2007).

In conclusion, this study has developed a novel method for demonstrating cerebellar inhibition that is individualized based on a participant’s individual, physiologically determined baseline CBI50 level. Clear dis-inhibition of cerebellar projections to M1 is seen at CBI50,
CBI50+5%, and CBI50+10% following a motor acquisition task that has been shown through previous fMRI studies to activate regions of the cerebellar cortex and cerebellar nuclei. With this method, each subject’s curve is made relative to a 50-percent baseline defined as CBI50 to allow comparability between subjects. This method is sensitive for studying neurophysiological changes that occur with motor learning and acquisition. A tool of this nature is important since it allows for comparisons of human cerebellar output and aids the study of human cerebellar physiology.

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References


Table of Figures

FIG. 1. Inhibition of the test MEP at increasing cerebellar stimulation intensities for a typical participant both before (A) and after (B) motor learning. Test MEPs are the dotted lines and produced with M1 stimulation only, and solid lines represent cerebellar + M1 stimulations.

FIG. 2. Examples of EMG traces with extraneous activity. A: Cervicomedullary evoked potential (CMEP) with a latency of approximately 18 ms. B: Cervical root activity with a latency of 14 ms. Any data traces with this activity were not included in analysis. Dotted lines represent test MEPs produced with M1 stimulations and solid lines represent cerebellar + M1 stimulations. Stimuli to the posterior fossa (PF) and motor cortex (M1) are labelled to show the temporal order.

FIG. 3. Representative data from three subjects at three RThs (42, 48, and 52% MSO). The CBI50 level is shown as a dotted line within a grey-shaded region. A: Subject’s data is related to stimulator output showing inhibition at variable regions of the x axis. B: Subject’s data is related to CBI50 which then allows for comparisons with group data at CBI50 and at levels adjacent to CBI50.

FIG. 4. Mean group responses related to CBI50, showing cerebellar inhibition at pre-intervention (filled circles) and post-intervention (open triangles). A: The experimental group shows significant changes following the task that possibly mean cerebellar output has changed. B: The control group shows no significant changes. Due to the small sample size, statistical analysis was not performed in this group. Error bars depict standard error.

FIG. 5. Performance measures following the 15 minute practice, showing improvements to both reaction time (A) and accuracy (B). Error bars depict standard deviation.
Cerebellar-M1 stimulations (ISI 5 ms)

M1 stimulations only
A

B

PF

M1

30 ms

500 µV

100 µV

30 ms
A

CBI50+10% CBI50+5% CBI50

Conditioned Response (%)

Stimulator Output (% Relative to CBI50)

- Pre Motor Learn
- Post Motor Learn

B

CBI50 CBI50+5% CBI50+10%

Stimulator Output (% Relative to CBI50)

- Pre Control
- Post Control

Conditioned Response (%)

CBI50 CBI50+5% CBI50+10%

Stimulator Output (% Relative to CBI50)