

Laterality Differences in Cerebellar–Motor Cortex Connectivity

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Lateralization of function is an important organizational feature of the motor system. Each effector is predominantly controlled by the contralateral cerebral cortex and the ipsilateral cerebellum. Transcranial magnetic stimulation studies have revealed hemispheric differences in the stimulation strength required to evoke a muscle response from the primary motor cortex (M1), with the dominant hemisphere typically requiring less stimulation than the nondominant. The current study assessed whether the strength of the connection between the cerebellum and M1 (CB–M1), known to change in association with motor learning, have hemispheric differences and whether these differences have any behavioral correlate. We observed, in right-handed individuals, that the connection between the right cerebellum and left M1 is typically stronger than the contralateral network. Behaviorally, we detected no lateralized learning processes, though we did find a significant effect on the amplitude of reaching movements across hands. Furthermore, we observed that the strength of the CB–M1 connection is correlated with the amplitude variability of reaching movements, a measure of movement precision, where stronger connectivity was associated with better precision. These findings indicate that lateralization in the motor system is present beyond the primary motor cortex, and points to an association between cerebellar M1 connectivity and movement execution.

Keywords: cerebellum, motor control, reaching, transcranial magnetic stimulation

Introduction

In everyday life, we do not use our hands symmetrically. Right-handed individuals tend to overuse the right arm, preferring it even when either arm is equally close to the target of a reaching movement (Oliveira et al. 2010). It has been suggested that this preferential use leads to better performance with the dominant arm due to the greater amount of practice, perhaps indicating more accurate state estimation and more sensitive feedback processing (Flowers 1975). While it is generally understood that each hand is primarily controlled by the contralateral motor cortex (M1), evidence from fMRI suggests that the left (dominant) hemisphere is often preferentially recruited when performing complicated movements with the left hand (Cramer et al. 1999; Verstynen et al. 2005). However, it is not known whether right-handedness is also associated with stronger, more active connections between the left motor cortex and supporting structures, such as the cerebellum.

The cerebellum is believed to be responsible for producing accurate movements among healthy individuals. Indeed, the hallmarks of cerebellar disorder are ataxia and dysmetria (Holmes 1917; Manto et al. 1994; Trouillas et al. 1997). Ataxia

is a disorder of coordination, and dysmetria is specifically a difficulty in making accurate discrete movements. Since laterality effects can be observed both behaviorally and by using brain imaging techniques such as fMRI, it is conceivable that laterality differences may also be present in the cerebellum and its connections to the motor cortex.

Cerebellar–Brain Inhibition (CBI) is a measurement of the strength of connectivity between the cerebellum and M1 (Ugawa et al. 1995; Pinto and Chen 2001; Daskalakis et al. 2004; Galea et al. 2009). As a physiological measure, it is sensitive to behaviors such as locomotor adaptation (Jayaram et al. 2011) and reach adaptation (Schlerf et al. 2012). The majority of studies have focused on the connectivity between the cerebellum and M1 in the dominant hand. Therefore, little is known about potential differences between the left and right hands, and how such differences might relate to behavioral differences. There is, however, evidence from other methodologies about cerebellar connectivity with the cerebrum. Buckner et al. (2011), in an impressive study, demonstrated functional interactions between the cerebellum and motor cortex using resting state connectivity measurements. They found, in general, a slightly larger number of voxels in the right cerebellar hemisphere connected with left M1 compared with the voxels in the left cerebellar hemisphere which connects to right M1. Importantly, both fMRI and fcMRI are correlative measures that lack information about the direction of information flow. Transcranial magnetic stimulation (TMS), in contrast, can provide a causal insight into the function of these anatomical connections.

In this study, we assessed the strength of cerebellar–M1 connectivity in both the dominant and nondominant hemispheres of healthy, right-handed individuals. We predicted that the dominant cerebellar hemisphere would more strongly inhibit M1 than the nondominant cerebellar hemisphere, due to the greater extent of voxels in this hemisphere connected with contralateral M1 (Buckner et al. 2011). We also explored whether the measured value of CBI would be related to behavior. To this end, in 2 separate sessions we asked participants to perform a reaching adaptation task. Cerebellar function is important for reach adaptation, as participants with damage to the cerebellum adapt poorly (Smith and Shadmehr 2005; Tseng et al. 2007; Werner et al. 2009; Criscimagna-Hemminger et al. 2010), stimulation over the cerebellum improves adaptation (Galea et al. 2011) as well as strengthening CBI (Galea et al. 2009). Cerebellar–M1 connectivity changes are associated with learning in the dominant hand and leg (Jayaram et al. 2011; Schlerf et al. 2012). As such, we predicted that adaptation performance may be related to the strength of the connection between the cerebellum and motor cortex. This prediction, taken in concert with our hypothesis that cerebellar

connectivity is lateralized, would suggest the existence of learning differences between the 2 arms. We performed this experiment to directly explore whether the resting connectivity between the cerebellum and M1 would be related to learning or other performance measures and assess laterality effects in these behaviors.

Materials and Methods

Participants

Fourteen healthy right-handed subjects (assessed using the Edinburgh Handedness Inventory) were recruited for Experiment 1 (7 female, mean age 27, SD = 7.5 years), with 9 of these individuals returning for the behavioral session. Following the completion of Experiment 1, we performed a corroboration analysis, recruiting 12 healthy right-handed individuals (5 female, mean age 23.5, SD = 2.5 years) to participate in Experiment 2. All participants provided informed consent, and all experimental procedures were approved by the Johns Hopkins Institutional Review Board (IRB) in accordance with guidelines set forth by the Declaration of Helsinki.

Experiment 1

We first characterized each individual's cerebellar-M1 connectivity using TMS. This was done while participants were seated comfortably in a chair, having completed no other behavioral experiment before or during the physiological assessment. Participants were then asked to return on separate days to complete 2 behavioral sessions, on days in which their brain physiology was not manipulated by stimulation.

During the TMS session, we used a 70-mm-diameter figure-of-eight coil (Bistim² stimulator, Magstim) to stimulate both left and right M1 to elicit motor evoked potentials (MEPs) of the FDI. Following a standard procedure, we first located the optimal scalp site in each hemisphere to activate this muscle. Our criteria require that the optimal site has the lowest motor threshold, defined as the smallest stimulator intensity required to generate MEPs of at least 50 μ V on 5 out of 10 pulses (Rossini et al. 1994). All MEPs were collected and measured using Signal software (Cambridge Electronic Designs, <http://www.ced.co.uk>), though for exploratory detection of the motor or brainstem thresholds the data were discarded at the end of the session. We saved the 2 optimal scalp locations in a frameless neuronavigation system so that we could return to them later (Brainsight, <http://www.Rogue-Research.com>). The position of the figure-of-eight coil was constantly monitored during stimulation to ensure spatially accurate stimulation throughout the experiment. We adjusted the strength of the pulses delivered to M1 such that we could elicit MEPs with average peak-to-peak amplitude of \sim 1 mV across 10 pulses. We assessed cerebellar excitability using a previously described protocol that measures cerebellar-M1 (brain) inhibition (CBI) (Ugawa et al. 1995; Ugawa 1999; Pinto and Chen 2001; Daskalakis et al. 2004; Galea et al. 2009; Schlerf et al. 2012). To avoid potential artifacts caused by antidromic stimulation of the pyramidal tract itself (Fisher et al. 2009), we first assessed the brainstem threshold. To this end, we searched for MEPs by stimulating directly at the midline (over theinion) with a double cone coil (diameter 110 mm) with the stimulator current directed downward (Ugawa, Uesaka et al. 1994). We asked subjects to preactivate both their left and right FDI by lifting the index finger, and searched for a stimulation intensity that evoked MEPs in either hand in 5 out of 10 pulses, with a maximal intensity cutoff of 80% of stimulator output (MSO). Using this procedure, we were able to measure a threshold in 7 out of 14 individuals. If we could not elicit MEPs with a maximal intensity of 80%, we then used 75% MSO as a reference intensity to minimize subject discomfort (Galea et al. 2009; Schlerf et al. 2012). Similar to Galea et al. (2009), we assessed CBI at 3 intensities in each hemisphere: 5%, 10%, or 15% less than the reference intensity or the brainstem threshold. For each measurement, a set of 20 TMS test stimuli (TS) were delivered to M1, with 10 pulses (selected at random) occurring 5 ms after a TMS conditioning stimulus (CS) over the contralateral cerebellum, centered \sim 3 cm lateral to theinion (Fig. 1A). This position was marked on

subjects' scalp using a felt pen to allow experimenters to return the coil to this location, as the double cone coil is easier to reliably maintain against the skull (unpublished data). For comparison, the remaining 10 TS were collected without a CS. For repeated measurements, the amplitude of the TS was adjusted to maintain an MEP of \sim 1 mV. To this end, 3–5 pulses are delivered prior to stimulation to check this measurement, and are not recorded for further analysis. This is a standard procedure for this type of experiment (Jayaram et al. 2011; Spampinato et al. 2011; Schlerf et al. 2012). CBI was computed as the ratio of the conditioned to unconditioned MEP. Pulses that did not result in an MEP (or pulses that resulted in an MEP $<$ 50 μ V) were excluded from analysis. This occurred very rarely: we never had to discard $>$ 2 pulses in a single 20 pulse measurement, and excluded $<$ 1% of all measured MEPs overall. While the stability and robustness of the CBI measurement might improve by collecting more pulses, our experience with this technique suggests that subject comfort can adversely be affected when a large number of pulses are applied over the cerebellum at a time. To keep our subjects comfortable, we have opted to deliver only 10 stimuli over the cerebellum. However, this pulse sequence has yielded effective results in the past (Galea et al. 2009; Jayaram et al. 2011; Schlerf et al. 2012). Although the spatial accuracy of TMS over M1 has been studied (Pascual-Leone et al. 1994), the accuracy of TMS within the cerebellum remains poorly understood. However, based on prior patient lesion studies, the CBI measurement is at the very least cerebellar hemisphere specific (Di Lazzaro et al. 1994; Ugawa et al. 1995), making it suitable for testing our hypotheses about laterality.

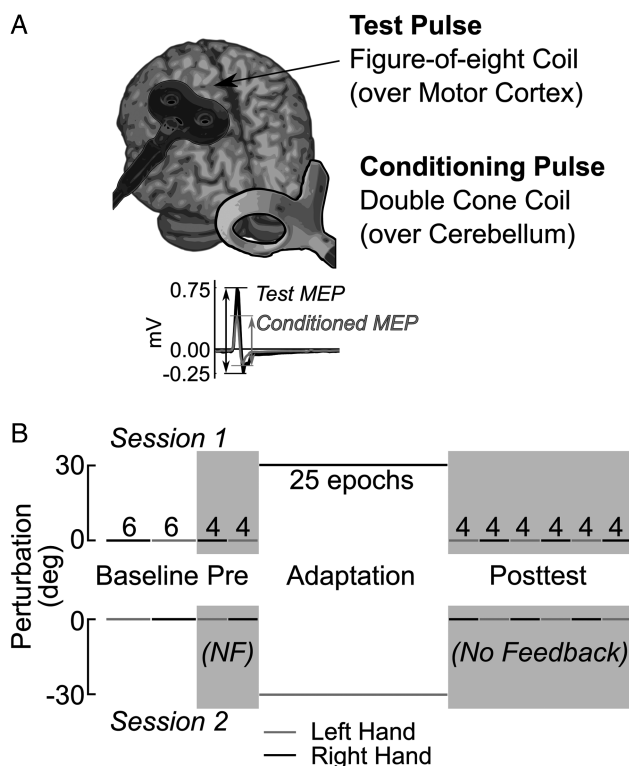


Figure 1. Methods overview. (A) An example of coil placement to measure CBI. If a conditioning pulse is delivered to the cerebellum prior to a pulse delivered over M1, the result is a smaller MEP (gray, below). (B) An example of the behavioral task used in Experiment 1. Participants performed 2 sessions, at least a week apart. In all sessions, participants produce baseline movements with each hand followed by a pretest in which participants move without feedback about angular error. Next, participants perform a learning test, completing 200 trials with a 30° (clockwise or counterclockwise) angular rotation. Next, participants perform a post-test, with no feedback about angular error. Participants alternate hands during this post-test, so that we can obtain a measurement of both transfer to the other hand as well as an aftereffect. Participants switch hands and perturbation directions across sessions.

Behavioral Task, Experiment 1

Participants who returned to participate in the behavioral task came in on 2 separate sessions, at least a week apart, and with at least one day separating the TMS and behavioral sessions. Participants were seated in a Kinarm exoskeleton robot (<http://www.bkin-tech.com>) to perform a center-out reaching task, making “slicing” movements to 8 targets spaced 10 cm away from a central location without view of their hands. The center was located at a point in line with the shoulder midpoint, at a position where both arms could comfortably reach it without encountering the mechanical stops of the Kinarm device. Once a participant’s hand moved >10 cm from the start location, the movement was considered “complete”. The target, which was originally cyan, changed color at the termination of the slice, turning red if participants were moving too quickly (faster than 200 ms), green if participants were moving too slowly (slower than 250 ms), and white if they were within the target range. In some blocks, participants were given feedback about their reach endpoint, as the cursor was frozen at this location. In other blocks, participants did not see their cursor during the movement and received no feedback about their accuracy.

Each behavioral session was broken down into 11 blocks (Fig. 1B). During the first block, participants performed at baseline, making 48 reaching movements (broken into 6 epochs of 8 trials each) with their “learning” arm (both the right or left arms served as the learning arm, with the order counterbalanced across sessions). Next, participants made 48 reaching movements to the same targets (in extrinsic space) with their “nonlearning” arm. The Pretest (first shaded region in Fig. 1B) consisted of the third and fourth blocks, each of which contained 32 reaches without receiving any endpoint feedback. The third block was completed with their learning arm, and the fourth with the nonlearning arm. Next participants completed the Adaptation block, performing 200 reaches with their learning hand, with the cursor displaced by a consistent angular rotation of 30° clockwise or counterclockwise (order balanced across sessions). Finally, participants performed a Post-test (second shaded region in Fig. 1B) consisting of 6 additional blocks of 32 reaches, each without feedback, alternating between their nonlearning and learning arms. During Session 2 (at least 1 week apart), participants performed an identical experiment, with the opposite arm serving as the learning arm.

We chose to require slicing movements (instead of terminal endpoint reaching) for 2 main reasons. First, this type of movement, especially when performed quickly, involves very few online corrections. As such, visual feedback is primarily important for knowledge of results (KR), and can be withheld to assess retention without requiring that participants adopt a different control regime (Shabbott and Sainburg 2010). Second, a slicing movement emphasizes control over the heading direction, which is the most sensitive movement parameter to a visuomotor rotation. Control over reach amplitude is not required by the task instructions, and amplitude variability has no impact on task success according to the feedback available to the subject. Importantly, previous investigations found very little difference between adaptation rates of healthy subjects under both terminal endpoint reaching and slicing movements (Tseng et al. 2007).

Experiment 2

To corroborate findings from Experiment 1, we conducted an analysis of an independent sample. The data represent a subset of a larger dataset collected to explore a different feature of the cerebellar response to behavior (Spampinato et al. 2011), although the analysis presented here is entirely independent of (and orthogonal to) those efforts. TMS was performed using similar procedures, ensuring that the cerebellar conditioning pulse was less intense than the brainstem threshold value, with a maximal intensity of 75% MSO. Instead of collecting separate intensities to explore the recruitment curve for CBI, however, a single intensity was used. In contrast to Experiment 1, stimulation intensity for the cerebellum was not held consistent across hemispheres for 2 participants, but rather adjusted (by repeated measurements) to obtain the strongest and most stable measure of cerebellar–M1 inhibition in both hemispheres independently while at rest. Otherwise, procedures were nearly identical, with CBI computed as the ratio of the average MEP (measured at FDI) from 10 conditioned

pulses to the average MEP from 10 unconditioned pulses. The strength of the pulse from the stimulator placed over M1 was adjusted to obtain an unconditioned MEP with an amplitude near 1 mV.

Behavioral Task, Experiment 2

Participants in Experiment 2 performed a similar center-out task, producing slicing movements to one of 8 targets. In contrast with Experiment 1, this task was performed on the same day as the stimulation, rather than in a separate session. Furthermore, instead of using the robotic device, participants controlled a cursor using a digitizing tablet (Wacom Intuos3). The stylus was secured to the index finger, and moved to both hands. When the movement was too slow, a low-pitched tone was provided as feedback. If the movement was too fast, a high-pitched tone was provided as feedback. This task and apparatus was very similar to that used in a recent study (Schlerf et al. 2012). Participants produced 96 movements with the right hand, followed by 96 movements with the left hand. The first 10 movements with either hand were discarded to allow participants to acclimate to the task instructions. No visuomotor perturbation was delivered during this period. Participants did subsequently receive a visuomotor perturbation; however, these data are not of interest to the current investigation and will not be analyzed here.

EMG Recording

We used surface electromyography (EMG) to measure the response to TMS. Subjects sat comfortably in a chair with both arms resting on a pillow placed on their lap. The skin was treated with a mild abrasive gel and then cleaned with isopropyl alcohol to reduce impedance. EMG activity was captured through a pair of disposable surface electrodes. Active electrodes were placed over the left and right first dorsal interosseous muscles (FDI), with the reference electrodes placed on the knuckle, ~3 cm away. Ground electrodes were placed over the head of the ulna, bilaterally. EMG signals were recorded, amplified, and filtered using a Viking IVP (Nicolet; bandwidth, 5 Hz to 1 kHz; Viasys Healthcare). Output from the Viking was sent to a CED 1401 (<http://www.ced.co.uk>), where Signal software (version 4.06) was used to time-lock the EMG response to TMS pulses and store the data for off-line analysis using custom scripts in MATLAB.

Data Analysis

All data were recorded for offline analysis on PC workstations running MATLAB. For the TMS analysis, we measured the amplitude of every MEP generated. For categorization and analysis, we recorded the relative intensity of the conditioning pulse relative to the reference value (either the brainstem threshold or 75% of MSO), whether the MEP was conditioned or unconditioned, and which muscle was being stimulated. If no MEP was visible on a particular pulse, that pulse was excluded from analysis. We assessed CBI statistically by subjecting the ratios to a repeated measures ANOVA (ANOVA_{RM}), with factors Laterality (in Experiments 1 and 2) and Intensity (in Experiment 1 only).

During the behavioral task, participants were provided with the endpoint error on every trial. This was defined as the difference between the angle between the target, the start location, and the cursor when it crossed the target distance. Trials in which the participant failed to move far enough on the first reach and then made a second, corrective reach were excluded from analysis. This occurred on ~0.3% of trials. We also measured the kinematic variables movement time and reach amplitude. Movement time was defined as the time the cursor spent traveling between the start region and the target distance. Reach amplitude was defined as the maximal distance reached by the participant. No feedback was provided about reach amplitude; participants had only to achieve a minimum value of 10 cm for the trial to be counted. We analyzed these kinematic and learning behaviors statistically using paired *t*-tests. As we were not certain that the data were normally distributed, we also performed permutation tests by repeatedly assigning datapoints at random to our conditions and evaluating the true difference against these other potential differences. We then performed linear correlations between behavioral measures and CBI, using the strongest (lowest ratio) measurement of CBI achieved for

each hemisphere. To protect against erroneous conclusions due to violations of normality, correlations were performed using both Pearson's R (which assumes normally distributed data) and Spearman's ρ (which is nonparametric).

Results

Experiment 1

CBI Recruitment Curves

We first assessed CBI at 3 different intensities in both hands. We found a stronger inhibition in the dominant side (from the right cerebellar cortex to the left motor cortex) relative to the nondominant side (Fig. 2). As expected, in both sides the amount of inhibition observed decreased as the intensity of the conditioning pulse was reduced. Note that to avoid differences in the 2 primary motor cortices we always stimulated M1 at an intensity sufficient to generate MEPs in the corresponding FDI of ~ 1 mV (see Supplementary Table 1).

We assessed the CBI recruitment curves statistically using 3×2 repeated-measures ANOVA, with factors Conditioning Pulse Intensity (5%, 10%, or 15% below brainstem threshold) and Laterality (Right or Left cerebellar hemisphere). We observed main effects of both Conditioning Pulse Intensity ($F_{2,26} = 21.2$, $P < 5e-6$) and Laterality ($F_{1,26} = 9.02$, $P < 0.02$) on CBI ratios, but no interaction ($F_{2,26} = 0.39$, $P > 0.6$). To determine whether CBI ratios differences were affected by M1 excitability, we subjected the MEP elicited by the test pulse to the same analysis. No significant main effects or interactions were observed (Conditioning Pulse Intensity, $F_{2,26} = 2.01$, $P > 0.15$; Laterality, $F_{1,26} = 0.05$, $P > 0.8$; Interaction, $F_{2,26} = 1.15$, $P > 0.35$).

Behavioral Measurements

A subset of 9 subjects returned to participate in a reach adaptation task, learning with each arm on separate days. We observed no overall difference in learning between the right and left arms (Fig. 3A,B). Participants, on average, learned to reduce their errors by 19.6 (SD = 1.8) degrees with their left arm, and 19.6 (SD = 4.1) degrees with their right arm ($t_{(8)} = 0.17$, $P > 0.8$; by 10 000 iteration permutation test, $P > 0.85$). Aftereffects (measured with the same arm) were 15.2

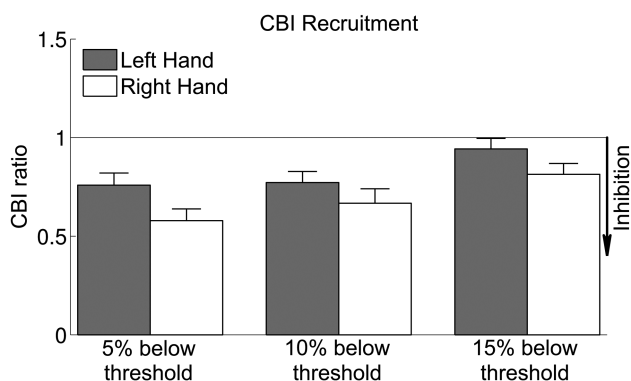


Figure 2. CBI Recruitment ($n = 14$). CBI at decreasing intensities relative to brainstem threshold is shown. As the difference between the stimulation intensity and brainstem threshold increases, the overall inhibition decreases, with the measured ratio approaching 1. The CBI ratio measured for the left hand (gray bars) is closer to 1 than the ratio measured for the right hand (white bars).

(SD = 3.7) degrees when learning with the left arm, and 14.2 (SD = 3.1) degrees when learning with the right ($t_{(8)} = 1.13$, $P > 0.2$; by 10 000 iteration permutation test, $P > 0.5$; Fig. 3C). We also tested transfer, looking at how much the new motor pattern influenced reaching in the untrained arm. We examined this by comparing the change in behavior of the right arm (always measured without feedback) caused by the adaptation of the left, and vice versa. Following left arm adaptation, the right arm changed performance by 2.5 (SD = 2.1) degrees, and following right arm adaptation the left arm changed performance by 2.2 (SD = 1.9) degrees. This difference was not significant ($t_{(8)} = 0.27$, $P > 0.7$; by 10 000 iteration permutation test, $P > 0.7$).

We also examined basic kinematic features of the reaching movement during the baseline blocks, before any perturbations were applied (Fig. 3D). This was chosen in case the presence of a perturbation changed kinematic features. Movement time (which was guided by colored feedback) was not systematically different across arms, as reaches took on

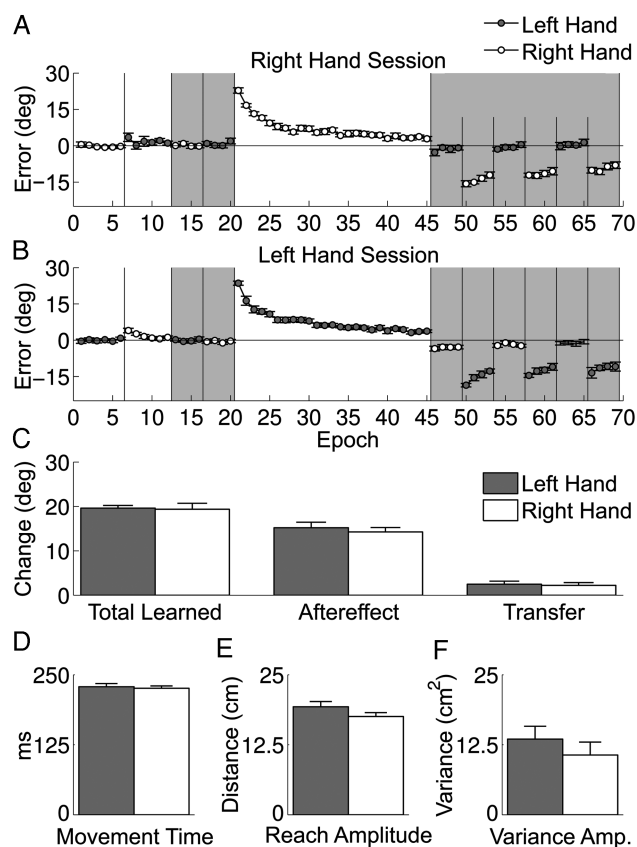


Figure 3. Behavioral features ($n = 9$). (A) The average learning performance during the session in which the right hand learns. Errors are presented as though the perturbation is always counterclockwise, although the real perturbation could be either clockwise or counterclockwise. (B) As in A, but showing the session in which the left hand learns. Gray circles represent epochs (averages of 8 trials) during which the left hand is moving, and open circles represent epochs during which the right hand is moving. (C) Summary bar graphs for the learning behavior, comparing the overall amount learned, the magnitude of the aftereffect, and the amount transferred to the opposite hand. (D) The average movement time (ms). (E) Shown is the overall reach amplitude, of which no feedback was provided. Participants reach slightly longer when moving with the left hand, although this difference was not significant after multiple comparison correction. (F) The variance of the reach amplitude. The difference is not significant. In C–F, shaded bars represent data from the session in which the left hand learns, and white bars represent data from the session in which the right hand learns.

average 228 ms (SD = 18) with the left arm, and 226 ms (SD = 12) with the right arm ($t_{(8)} = 0.5$, $P > 0.6$; by 10 000 iteration permutation test, $P > 0.7$). Reach amplitude did differ slightly, with movements tending to be slightly longer when reaching with the left arm. We asked participants to make slicing movements, and as such they received no feedback about the reach amplitude. On average, participants made 19.3 (SD = 2.8) cm reaches with their left hand, and 17.5 (SD = 2.1) cm reaches with their right hand (Fig. 3E). This difference was significant at uncorrected levels ($t_{(8)} = 3.02$, $P = 0.017$). However, since this analysis was exploratory in nature and we performed 3 total tests on kinematic features, this did not pass a Bonferroni corrected alpha of 0.0167, though it did approach significance even at this conservative threshold. Using a nonparametric measurement, however, failed to corroborate this result (by 10 000 iteration permutation test, $P > 0.15$). Nevertheless, this was the most notable difference across hands. The variance of reach amplitude, a measure that describes precision, did not differ strongly across hands, with a variance of 13.5 (SD = 6.9) cm^2 when reaching with the left hand and 10.7 (SD = 6.9) cm^2

when reaching with the right hand. This difference was not significant ($t_{(8)} = 1.76$, $P = 0.12$; by 10 000 iteration permutation test, $P > 0.3$).

Correlations between Physiology and Behavior

Since we used the same participants for both brain stimulation and behavior, we explored whether CBI measured at rest was itself predictive of any behavioral measurements. Figure 4 shows relationships between the maximal CBI value observed during the stimulation session and the behavioral variables plotted in Figure 3. CBI was not correlated with the total amount learned ($r = -0.06$, $P > 0.8$; $\rho = 0.06$, $P > 0.8$), the size of the aftereffect ($r = 0.26$, $P > 0.25$; $\rho = 0.34$, $P > 0.15$), transfer to the opposite hand ($r = -0.17$, $P > 0.4$; $\rho = -0.5$, $P > 0.8$), movement time ($r = 0.04$, $P > 0.8$; $\rho = 0.14$, $P > 0.5$), or reach amplitude ($r = 0.33$, $P = 0.18$; $\rho = 0.34$, $P = 0.17$). However, there was a strong relationship between CBI and the variance of reach amplitude ($r = 0.62$, $P < 0.006$; $\rho = 0.70$, $P < 0.002$), with stronger (lower) values of CBI associated with less

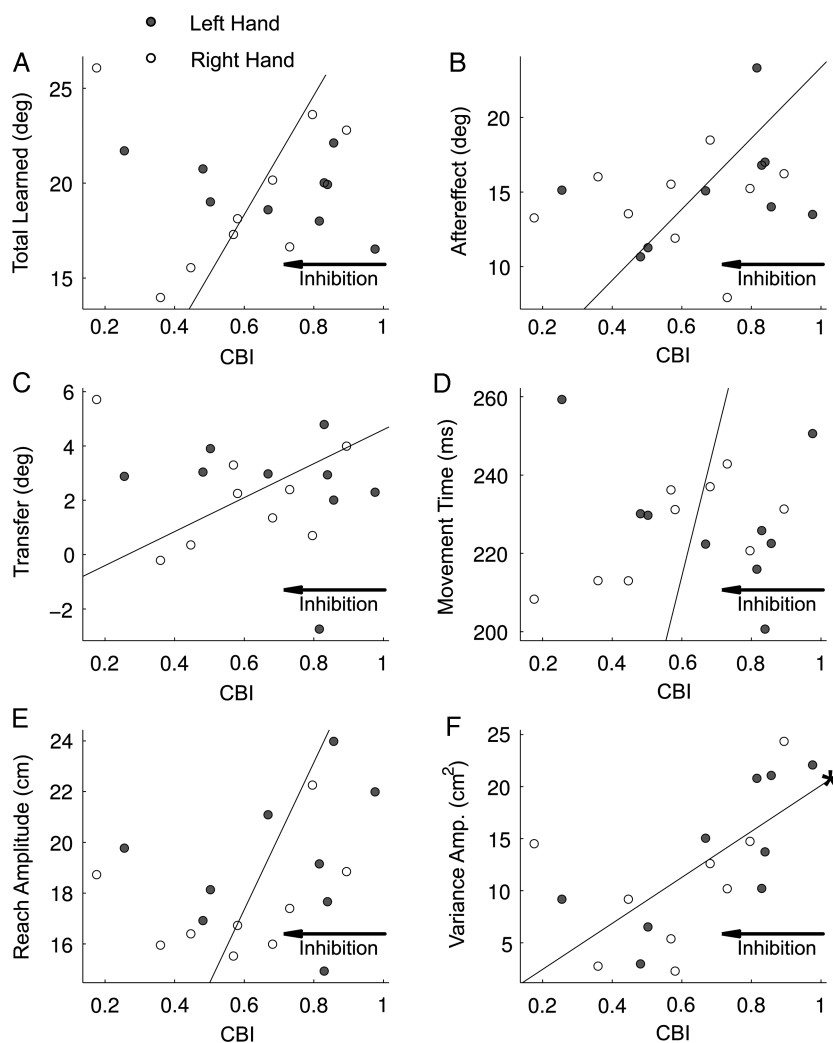


Figure 4. Correlations between CBI and behavior. (A) The relationship between the strongest CBI ratio observed and the total amount learned (assessed at the end of Block 5). (B) The relationship between CBI and the size of the aftereffect (assessed in Block 7). (C) The relationship between CBI and the amount of learning which was transferred to the opposite hand (assessed in Block 6), measured in extrinsic coordinates. (D) The relationship between CBI and movement time. (E) The relationship between CBI and mean reach amplitude (measured in Blocks 1 and 2) (F) The relationship between CBI and the variability of reach amplitude (measured in Blocks 1 and 2). This correlation was significant ($r = 0.62$, $P < 0.006$; $\rho = 0.70$, $P < 0.002$), even after correcting for multiple comparisons.

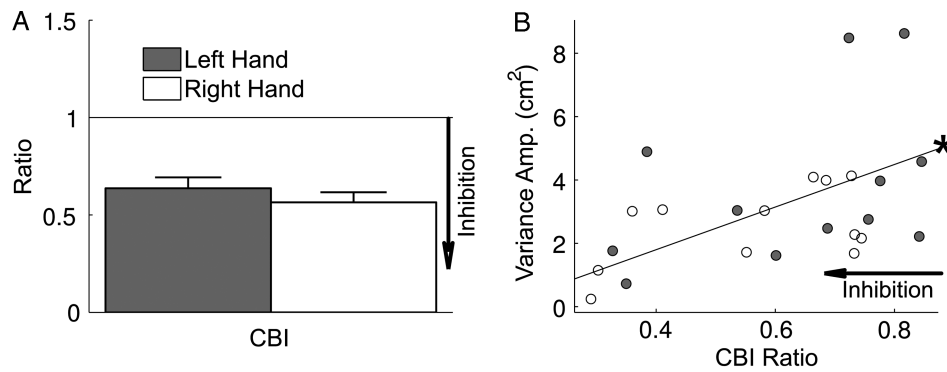


Figure 5. Corroboration study. (A) The difference in CBI for the right and left sides in an independent cohort. While there was a trend for CBI over the right cerebellar hemisphere to be stronger, this factor was not significant. However, the intensity of stimulation was not constant. (B) The relationship between the CBI ratio and the variance of the reach amplitudes (assessed following 90 reaches of each hand) in an independent cohort. The correlation is significant ($r = 0.46$, $P < 0.025$; $\rho = 0.42$, $P < 0.05$).

amplitude variability (Fig. 4F). This relationship exceeds a Bonferroni corrected alpha of 0.0083.

Experiment 2, Corroboration with Independent Subjects

Since we conducted multiple correlations between physiology and behavior, and had a relatively small sample size, we elected to conduct a corroboration analysis of an independent dataset to confirm the observed relationship between measured CBI and measures of reach amplitude in slicing movements (Fig. 5). In an independent dataset ($n = 12$), with participants making slicing movements on a drawing tablet, we found that average reach amplitude, which approached significance in Experiment 1, was influenced by laterality. Participants reached slightly further with their left hand (15.78 cm, $SD = 1.46$ cm) than with their right (14.37 cm, $SD = 0.87$ cm), which proved to be a significant difference in this experiment ($t_{(22)} = 2.876$, $P < 0.01$). In addition, we found a significant correlation between CBI and reach variance ($r = 0.46$, $P < 0.025$; $\rho = 0.42$, $P < 0.05$). This relationship was very similar to Experiment 1, with stronger CBI (lower ratio) associated with less endpoint variance across movements. This design also allows us an opportunity to partially replicate the laterality difference in CBI (Fig. 2), with the caveat that the CBI pulse intensities were not constant across hemispheres, but adjusted to obtain the strongest measure of cerebellar–M1 inhibition (see Materials and methods). As such, there was a trend for participants to have less CBI (higher ratios) with the left hand than the right, but this trend was not significant across the population tested ($t_{(11)} = 1.228$, $P > 0.2$; by 10 000 iteration permutation test, $P > 0.35$). Altogether these results further corroborate the findings in Experiment 1.

Discussion

The most striking result from this study is the observation that the right cerebellum exerts a stronger inhibitory influence over M1 than the left cerebellum. Since all participants were right handed, we explored whether this lateralized physiological response was related to behavior. In a study of visuomotor reach adaptation, we observed no strong differences in learning between dominant and nondominant arms. In addition, a physiological measure of cerebellar–M1 connectivity obtained at baseline did not predict any differences in learning. However, we did observe a nearly significant laterality effect on the amplitude of the reaching movements (which reached

statistical significance in Experiment 2), a kinematic measurement related to movement execution but not adaptation. Furthermore, we found that the strength of CBI was correlated with the variability or precision of these measurements, where more consistent movement amplitude was observed on those subjects with higher CBI. This was a robust effect that was also observed in a corroboration analysis in an independent dataset.

The cerebellum has been long recognized as a critical structure involved in motor control. Indeed, a hallmark of cerebellar dysfunction is dysmetria, an inability to make precise, accurate movements (Holmes 1917; Manto et al. 1994; Trouillas et al. 1997). The observed correlation between CBI and amplitude variance suggests that a stronger connection between the cerebellum and M1 results in more accurate endpoints, and better precision across multiple movements, which are broadly along the idea that a damaged cerebellum results in less accurate endpoints. This is consistent with prior investigations which described that individuals with cerebellar ataxia show less CBI than healthy controls (Ugawa, Genba-Shimizu et al. 1994). Altogether these observations suggest that the cerebellar inhibition over motor cortex is not merely present or absent, but rather the strength of this connection is important and behaviorally relevant. Thus, manipulation of these connections might have behavioral consequences.

Measuring the variance of reach amplitude in a slicing paradigm is not common practice. We believe, however, that it provides qualitatively similar insight into processes often referred to as “implementation variance,” which are typically parameter estimates requiring thousands of trials (Cheng and Sabes 2006, 2007). Furthermore, as a direct measurement of the inherent precision of motor execution, it is relatively pure. Since feedback about reach amplitude is typically withheld, there are fewer potential confounds introduced by trial-to-trial fluctuations compared with direction variability, sometimes used as a surrogate for plant variance (Schlerf et al. 2013). Thus, the measurement can be repeated over many trials for a reliable assessment. While the current experiment was not designed to facilitate this analysis, future experiments could further validate this measurement by comparing it to the implementation variance computed through other methods, or perhaps analyzing the relationship between this measurement and trial-by-trial learning rates (Cheng and Sabes 2006, 2007). In the current study, we observed a robust correlation between CBI and amplitude variability. Initially, this emerged from an

exploratory analysis. However, it did pass a Bonferroni correction, which is a standard and conservative correction for multiple comparisons. Furthermore, we conducted a corroboration analysis in an independent dataset, and still observed a reliable correlation. Thus, it appears that this measurement is consistent enough to be meaningful, despite not being commonly reported. Of note, we also observed a strong trend, which was later corroborated in the second experiment, of larger amplitude movements in the nondominant hand. Altogether, these results point to an association between the observed laterality in the cerebellar–M1 connectivity and kinematics measures during reach movement execution (not learning).

The physiological nature of the relationship between movement precision and cerebellar–M1 connectivity is of interest. While the current study does not directly address this, there are certain parallels that can be drawn to other studies. Previous investigations have observed that the cerebellum responds by decreasing its inhibition over M1 when movement corrections are required to respond to environmental changes (Jayaram et al. 2011; Schlerf et al. 2012). Mechanisms similar to long-term depression (LTD), where the response of Purkinje cells to a particular stimulus (conveyed by Mossy Fiber input) is decreased after an error signal (conveyed by Climbing Fibers), appear to be the most important neurophysiological processes underlying the cerebellar contribution to motor learning and adaptation (Ito 1998; Christian and Thompson 2003; Medina and Lisberger 2008). Previously observed TMS results which suggest that learning causes a decrease in cerebellar excitability are consistent with this neurophysiological interpretation (Jayaram et al. 2011; Schlerf et al. 2012). Interestingly, when movement continues in the adapted state, CBI returns to baseline values, suggesting some sort of encoding process (Schlerf et al. 2012). A popular interpretation of this phenomenon is that the cerebellum contributes to form or store an internal model, which is updated through error-based learning and LTD-like processes (Tseng et al. 2007; Shadmehr et al. 2010; Jayaram et al. 2011). While superior fine motor control is not necessarily learned, it would be reasonable to suggest that a strong internal model is a contributing factor to more precise motor control. The correlation between low amplitude variability and higher CBI would be consistent with this framework, particularly if one makes the assertion that strong CBI is indicative of a strong internal model.

It was somewhat surprising that, despite finding a difference in cerebellar connectivity, we observed no difference in adaptation between arms, a process which involves the cerebellum (Martin et al. 1996; Galea et al. 2011; Jayaram et al. 2011, 2012; Schlerf et al. 2012). Early work on interlimb differences suggested that the right arm should have stronger sensory information and better feedback control (Flowers 1975), which is typically associated with faster learning (Burge et al. 2008; Shabbott and Sainburg 2010). However, we found no observable difference. This may have been due to our use of a ballistic slicing task, which has been found to be a less demanding task than other point-to-point tasks (Tseng et al. 2007), and thus performance may already be at ceiling. It may also be the case, however, that the 2 arms simply always adapt at a similar rate (Galea and Miall 2006), or that the visuomotor adaptation paradigm itself is more sensitive to a limb-independent process which remains equally active regardless of the arm used (Simani et al. 2007).

It is important to point out that the observed correlations between CBI and behavioral precision do not speak to causality. It is tempting to suggest that the observed weaker inhibition by the cerebellum may underlie the preference for dominant-hand movements observed in other studies (Oliveira et al. 2010). However, it is also possible that stronger cerebellar connectivity is a practice effect, which arises from the use preference in the first place. Such a hypothesis is beyond the scope of the current data, though it is testable. Participants could be required to use their nondominant hands preferentially for a long period of time, with cerebellar–M1 connectivity assessed before and after this practice. The relationship could be further characterized by assessing whether enhancing cerebellar–M1 inhibition through procedures such as transcranial direct current stimulation (Galea et al. 2009) might be able to improve performance during restricted-use experiments. Importantly, a better understanding of the causal relationship between cerebellar connectivity and motor execution may have implications for the recovery of function following stroke.

Supplementary Material

Supplementary material can be found at: <http://www.cercor.oxfordjournals.org/>.

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Notes

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