NeuroImage 98 (2014) 147–158

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Contributions of the cerebellum and the motor cortex to acquisition and retention of motor memories

David J. Herzfeld [a,1], Damien Pastor [a,b,c,1], Adrian M. Haith [a], Yves Rossetti [b,c], Reza Shadmehr [a,*], Jacinta O'Shea [b,c,d]

[a] Laboratory for Computational Motor Control, Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[b] Lyon Research Center of Neuroscience, ImpAct team, INSERM U1028, CNRS UMR5292, Lyon 1 University, 69676 Bron, France
[c] Plate-forme Mouvement et Handicap, Hospices Civils de Lyon, Centre de Recherche en Neurosciences de Lyon, 69230, Saint-Genis Laval, France
[d] FMRIB, Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, UK

ARTICLE INFO

Article history:
Accepted 29 April 2014
Available online 9 May 2014

Keywords:
tDCS
Adaptation
Reaching
Consolidation
Retention
Generalization
Force field
Motor control

ABSTRACT

We investigated the contributions of the cerebellum and the motor cortex (M1) to acquisition and retention of human motor memories in a force field reaching task. We found that anodal transcranial direct current stimulation (tDCS) of the cerebellum, a technique that is thought to increase neuronal excitability, increased the ability to learn from error and form an internal model of the field, while cathodal cerebellar stimulation reduced this error-dependent learning. In addition, cathodal cerebellar stimulation disrupted the ability to respond to error within a reaching movement, reducing the gain of the sensory-motor feedback loop. By contrast, anodal M1 stimulation had no significant effects on these variables. During sham stimulation, early in training the acquired motor memory exhibited rapid decay in error-clamp trials. With further training the rate of decay decreased, suggesting that with training the motor memory was transformed from a labile to a more stable state. Surprisingly, neither cerebellar nor M1 stimulation altered these decay patterns. Participants returned 24 hours later and were re-tested in error-clamp trials without stimulation. The cerebellar group that had learned the task with cathodal stimulation exhibited significantly impaired retention, and retention was not improved by M1 anodal stimulation. In summary, non-invasive cerebellar stimulation resulted in polarity-dependent up- or down-regulation of error-dependent motor learning. In addition, cathodal cerebellar stimulation during acquisition impaired the ability to retain the motor memory overnight. Thus, in the force field task we found a critical role for the cerebellum in both formation of motor memory and its retention.

Introduction

When we interact with a novel object, we learn through trial and error to control that object, producing a motor memory that can be recalled the next time the object is encountered. Force field learning has been used as an experimental paradigm to uncover some of the processes that the brain relies on to accomplish this feat. In a typical experiment, the participant holds the handle of a robotic arm and makes a reaching movement, experiencing novel forces that displace the hand, resulting in error. This error engages short- and long-latency feedback pathways, producing a within-movement motor response to the error. In the subsequent reach the brain predicts some of the novel forces from the onset of the movement, resulting in partial compensation for the robot-induced forces. This trial-to-trial change in the motor commands has a specific pattern: the within-movement error feedback response is shifted earlier in time to produce a predictive response (Thoroughman and Shadmehr, 1999). With training, some of the modifications to the motor commands become a motor memory, as exemplified by the observation that the memory is disengaged when the robot handle is released (Kluzik et al., 2008), and is recalled days (Criscimagna-Hemminger and Shadmehr, 2008; Joiner and Smith, 2008) or months (Shadmehr and Brashers-Krug, 1997) later when the robot handle is grasped.

Formation of this motor memory appears independent of human medial temporal lobe structures (Shadmehr et al., 1998), but dependent on the integrity of the cerebellum (Criscimagna-Hemminger et al., 2010; Donchin et al., 2012; Smith and Shadmehr, 2005), and the motor cortex (Arce et al., 2010b; Li et al., 2001; Orban de Xivry et al., 2011a, 2011b, 2013; Richardson et al., 2006). In particular, a study in humans demonstrated that reversible disruption of the thalamic projections of the cerebellum to the cortex produced within-subject impairments in the ability to learn the force field task (Chen et al., 2006). Therefore, the current evidence points to the cerebellum as one of the structures that plays a critical role in the acquisition of this motor memory.

Here, we used transcranial direct current stimulation (tDCS) to alter function of the cerebellum and quantified the effect of this disruption on

* Corresponding author at: 410 Traylor Building, Johns Hopkins School of Medicine, 720 Rutland Ave, Baltimore, MD 21205, USA.
[1] D.J.H. and D.P. contributed equally to this research.

http://dx.doi.org/10.1016/j.neuroimage.2014.04.076
1053-8119/

the ability to learn the force field task. tDCS of the cerebellum is thought to affect the excitability of Purkinje cells (Galea et al., 2009). Anodal cerebellar stimulation, which is thought to elevate the excitability of Purkinje cells, has been shown to increase rates of adaptation in visuomotor (Block and Celnik, 2013; Galea et al., 2010) and gait (Jayaram et al., 2012) tasks, whereas cathodal cerebellar stimulation, which is thought to reduce Purkinje cell excitability, has been shown to decrease rates of gait adaptation (Jayaram et al., 2012). By contrast, anodal stimulation of the motor cortex (M1) had no effect on the rate of visuomotor adaptation, the size of after-effects, or the rate of de-adaptation upon removal of the perturbation (Galea et al., 2011). However, immediately after adaptation and removal of anodal M1 tDCS, those in the stimulation group showed a reduced rate at which the resulting memory decayed in the absence of visual feedback (Galea et al., 2011). These findings led Galea et al. (2011) to propose that whereas the cerebellum may be critical for learning from error, the motor cortex plays a role in retention of the resulting memory. By contrast with the findings of Galea et al. (2011), Hunter et al. (2009) applied anodal stimulation to the motor cortex in a force field task and observed a larger reduction in signed kinematic errors during adaptation than in a sham tDCS condition, suggesting that motor cortical stimulation increased learning from error. Therefore, whereas current evidence suggests that stimulation of the human cerebellum can affect learning from error, it is unclear whether stimulation of the motor cortex affects learning from error and/or retention.

Here, we compared the effects of cerebellar and M1 stimulation on the process of acquisition and retention of motor memories in a force field paradigm. Given previous observations in other motor learning paradigms, we expected that M1 stimulation would not affect the rate of learning from error, whereas anodal cerebellar stimulation would increase this rate and cathodal cerebellar stimulation would decrease the rate of learning. In addition, to specifically test the hypothesis that anodal stimulation of M1 enhances retention of motor memories (Galea et al., 2011), we tested the effects of M1 anodal stimulation on both short-term retention (via blocks of error-clamp trials during the training blocks), and long-term retention (at 24 hours following completion of training).

Materials and methods

Fifty healthy self-reported right-handed volunteers (21 females; mean age ± STD of 24 ± 4.7 years, range 18–38 years) with no known neurological or psychiatric disorders participated in our study. All participants were naive to the purpose of the experiment and gave written informed consent. The study was approved by the Johns Hopkins School of Medicine Institutional Review Board. Participants were screened prior to enrollment in the study to ensure that they did not have conditions that would exclude them from a brain stimulation study (cardiac pacemakers, history of seizure, or aneurysm clips). Participants were also screened to ensure that they were not taking any neurological drugs.

Experiment 1: cerebellar stimulation

We recruited $n = 37$ participants for this experiment. They were divided into three groups: sham ($n = 12$), anodal cerebellar ($n = 15$), and cathodal cerebellar ($n = 10$) stimulation. During analysis of the data we noted that one participant in the cerebellar cathodal group exhibited large errors during field trials and failed to compensate for the forces over the course of the experiment. Although it is possible that this is related to the stimulation (as we will see, cathodal stimulation impaired the ability to learn), to err on the side of caution, the data from this participant were not included in our report.

tDCS (2 mA, 25 min) was delivered by a Phoresor II device (model PM850, IOMED) through two 5 × 5 cm saline-soaked sponge electrodes (Ferrucci et al., 2008; Galea et al., 2009, 2011). The current density was approximately 0.08 mA/cm^2. For the anodal tDCS group, the anode was centered on the right cerebellar cortex, 3 cm lateral to the inion (Galea et al., 2009; Ugawa et al., 1995), with the cathode positioned on the right buccinator muscle (i.e. on the cheek) (Galea et al., 2009, 2011). For the cathodal group the electrode polarity was reversed such that the cathode was placed over the right cerebellar cortex.

The procedures for the sham group were identical to the other groups. Anode and cathode positions were counterbalanced between cerebellum and buccinators. The current was increased over a period of 30 sec and then decreased back to zero. With this procedure, participants are unable to reliably distinguish real from sham stimulation (Gandiga et al., 2006; Kaski et al., 2012).

Both the experimenter and the participant were blind to the type of stimulation, as a third person uninvolved in the experiment controlled the tDCS settings. As illustrated in Fig. 1A, stimulation began with block n2 and concluded with block g2, lasting no more than 25 min. Brain stimulation was applied on Day 1 only. On Day 2, all participants performed block b1. Additionally, block b2 was performed by a subset of participants: $n = 12/12$ anodal cerebellar, $n = 10/12$ sham, and $n = 8/10$ cathodal.

Experiment 2: motor cortex stimulation

To determine whether the effects observed with anodal stimulation of the cerebellum were unique to this structure, or could also be elicited via anodal stimulation of the motor cortex, $n = 14$ additional participants were recruited. They performed the identical experiment during anodal tDCS of left M1 (2 mA, 25 min, 5 × 5 cm electrodes, induced current density of 0.08 mA/cm^2). The anode was positioned on the scalp overlying the "motor hotspot" of the right first dorsal interosseus (FDI) muscle, that is, the optimal position at which a consistent motor evoked potential, as recorded via EMG, could be elicited using minimal intensity transcranial magnetic stimulation (70 mm coil coupled with a Magstim 200). We used FDI (rather than biceps) muscle to localize M1, primarily because it is more easily activated via TMS. The size of the tDCS electrode (25 cm^2) makes it likely that coverage included both muscle representations. The other electrode was positioned on the skin overlying the contralateral supraorbital region.

Behavioral procedures

All volunteers participated in a standard force field task (Shadmehr and Mussa-Ivaldi, 1994). Using the right hand, each participant held the handle of a manipulandum and made center-out movements to a target (1 cm diameter, Fig. 1). The reach was perturbed by a velocity dependent clockwise curl force field that pushed the hand perpendicular to the direction of motion: $f = B\dot{x}$ where f is force on the hand, $B = [0, 13; -13, 0]$ N · s/m, and $\dot{x}$ is hand velocity. In the starting posture, the hand was positioned such that the shoulder and elbow were at 45° and 90° respectively (Fig. 1). Participants were unable to see their hand, which was occluded by an opaque horizontal screen. Instead, visual feedback regarding hand position was provided by a cursor (0.5 cm diameter) that was continuously projected onto the horizontal screen.

On each trial (except generalization trials, see below), one of the two targets appeared on the screen (pseudo-randomized with equal probability). Targets 1 (T1) and 2 (T2) were positioned at 10 cm at 135° and 315° (Fig. 1). The trial was successful if the hand arrived at the target within 400–500 ms after movement onset, with success indicated by an "explosion" of the target (an animation). Feedback regarding movements that were too fast or too slow was indicated via changes in target color. After completion of the trial, the robot brought the hand back to the start position. Participants were instructed to maximize the number of successful trials.

In some trials, an "error-clamp" was applied (Scheidt et al., 2000). In these trials, the force field was turned off. Normally, removal of the field

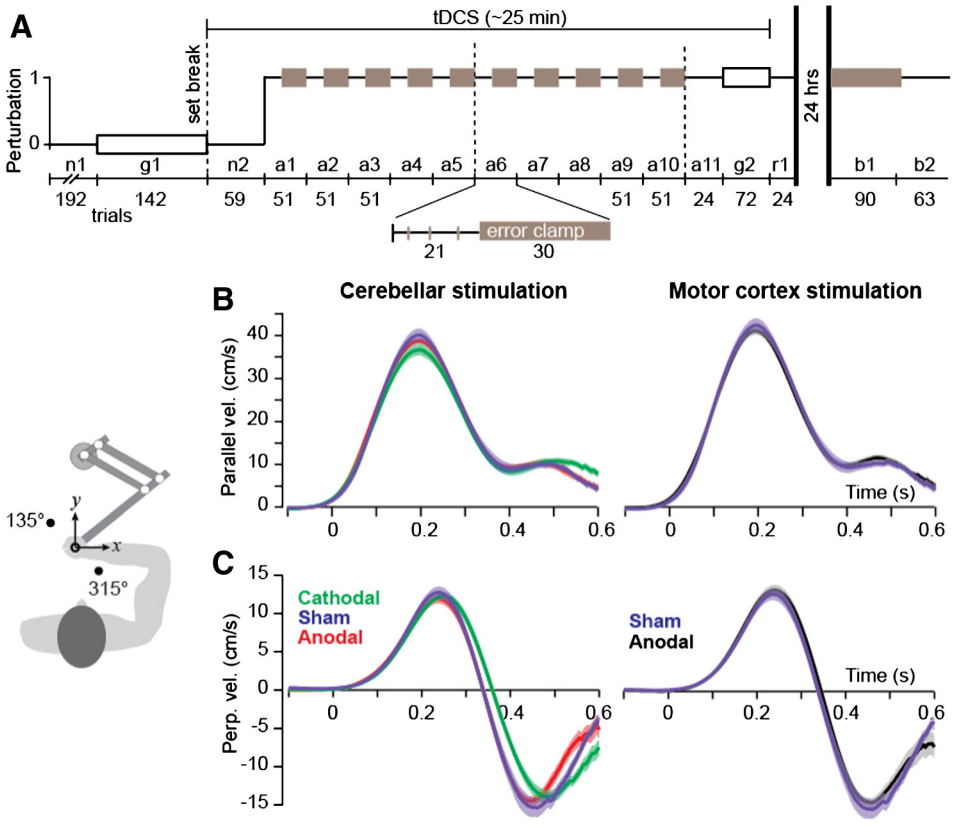

Fig. 1. Experiment protocol and effects of stimulation on feedback control. **A.** Volunteers were instructed to hold the handle of a manipulandum and reach to one of two targets that appeared at 10 cm. After a period of null field training (no perturbation, blocks n1 and n2), a clockwise curl force field was introduced. During training, short blocks of field trials were followed by short blocks of error-clamp trials (blocks a1–a11). Blocks g1 and g2 refer to trials in which generalization of learning was assayed at nearby targets. Block r1 provided relearning after block g1. The right cerebellum or the left motor cortex was stimulated during Day 1. Retention was assessed on Day 2 by means of a block of error-clamp trials (b1) followed by a block of re-exposure to the field (b2). Dashed lines are set breaks (around 1 min). B. Hand velocity parallel to the direction of target during stimulation of the right cerebellum or the left motor cortex, first 10 trials of block a1. C. Hand velocity perpendicular to the direction of target, first 10 trials of block a1. Cathodal stimulation of the cerebellum slowed the error-feedback response, indicated by the later time at which the perpendicular velocity trace crosses zero. Trajectories during anodal stimulation of the motor cortex or cerebellum were indistinguishable from the sham group. Data are mean ± SEM.

produces an after-effect. However, in error-clamp trials the hand path was constrained to a straight line to the target via stiff walls (spring coefficient 2000 N/m, damping coefficient 25 N·s/m). The stiff walls allowed us to measure the forces that the participant produced, serving as a proxy for the motor output that the brain generated in order to compensate for the force field expected from the robot.

The experiment was conducted over two consecutive days (Fig. 1A). On Day 1, the session began with two blocks of training in the null field without brain stimulation. Block n1 consisted of 192 trials to targets T1 and T2, including 48 interspersed error-clamp trials. Block g1 consisted of 142 trials to targets at ±45°, 90°, 112.5°, ±135°, 157.5°, 180°, and 225°. Brain stimulation was started at the onset of block n2. This was followed by another block of null field training (59 trials, including 15 error-clamp) to targets T1 and T2 (block n2). Participants then experienced alternating field and error-clamp blocks (labeled a1–a11). As illustrated in Fig. 1A, each of these blocks consisted of 21 field trials with 3 randomly inserted error-clamp, followed by 30 trials of error-clamp. Block a11 consisted of 24 field trials (including 5 error-clamp).

During blocks a1–a11, participants alternated between short blocks of field and error-clamp trials. This enabled measurement of two distinct properties of learning: 1) in field trials we assayed error-dependent learning by quantifying how the motor output changed from one trial to the next as a function of error, and 2) in error-clamp trials we assayed the stability of the developing memory by quantifying how the motor output decayed within blocks in the absence of error (Criscimagna-Hemminger et al., 2010; Smith et al., 2006).

Training on Day 1 concluded with 72 generalization trials (block g2, including 36 error-clamp) in which we quantified motor output to locations near the trained targets. The generalization targets were at ±22.5, ±45, and ±90° with respect to the training target T1. The reaches to the generalization targets were always in error-clamp. The generalization block consisted of cycles in which there was one movement to T1, followed by error-clamp movements to successive generalization targets chosen randomly so that every cycle included one of each of the target positions.

Following the generalization trials, we concluded Day 1 training with 24 trials in a re-learning block to targets T1 and T2 (block r1, including 5 error-clamp). At the end of Day 1 participants ranked their level of attention (1: least attentive, 7: most attentive), fatigue (1: least fatigued, 7: most fatigued), and perceived head discomfort (1: no discomfort, 7: extreme discomfort/pain) using a visual scale.

Retention of the motor memory was assessed on Day 2 by means of an error-clamp block (b1, 90 trials), followed by re-exposure to the field (b2, 63 trials, including 9 error-clamp). Stimulation was not applied on Day 2. All procedures were identical to between the two experiments, with the exception that on Day 2, all M1 participants were tested in block b1, but not b2.

Data collection and statistical analysis

A force transducer measured the forces applied by the participant at the robot handle and optical encoders measured position of the robot. The sensors and transducers were sampled at a rate of 200 Hz. Movement onset was defined as the time when the reach exceeded 10% of the maximum velocity in the direction of the target. Data from aborted trials, trials in which participants moved in the wrong direction

(exceeding 0.02 m from a line connecting the starting position and the target), and trials in which hand velocity did not exceed 0.08 m/s were excluded (<4% of all trials). Using these criteria, the following percentage of trials was removed prior to analysis: 3.8% (cerebellar anodal), 4.7% (cerebellar cathodal), 3.7 % (M1 anodal) and 3.7% (sham). All other trials were included in the analysis.

For each participant, the force profile measured during error-clamp trials in baseline block n2 was subtracted from error-clamp trials during adaptation. To quantify how well the forces that participants produced matched the perturbation forces, we computed a force index: the force $f(t)$ produced by the participant in an error-clamp trial was compared to the ideal force $f^*(t) = B\dot{x}(t)$ (field strength times the hand velocity) by finding the coefficient α that minimized the following:

$$\sum_{t=0}^{T} \left(\alpha f^*(t) - f(t)\right)^2. \quad (1)$$

In Eq. (1), T is time at end of the reach. We will refer to α that minimizes Eq. (1) as the force index.

Statistical analyses were performed using R (R-project, Vienna, Austria). Motor adaptation studies often show changes in the across-participant variance of the learned parameter as the experiment progresses. This implies that the covariance between two pairs of samples changes over the course of the experiment, violating the compound symmetry assumption of repeated measures ANOVA. Therefore, we used the generalized linear model (GLM) feature of R (gls) to test for fixed effects of block, stimulation type, and block by stimulation interactions (Laird and Ware, 1982). We constructed three models with different covariance structures, including compound symmetry, similar to the statistical model used for repeated measures ANOVA, autoregressive, and unstructured correlations. We compared the fit of these models using Akaike's information criteria (Akaike, 1974) and noted that an autoregressive structure provided the best fit in all tested cases. This autoregressive correlation structure assumed that consecutive measurements had a correlation given by the product of the measured variance and the discounting parameter, ρ, where $\rho \leq 1$. Therefore, the correlation between any two within participant measurements decreased as the temporal distance between the measurements increased:

$$\Sigma = \begin{pmatrix} \sigma^2 & \sigma^2\rho & \cdots & \sigma^2\rho^n \\ \sigma^2\rho & \sigma^2 & \cdots & \sigma^2\rho^{n-1} \\ \vdots & \vdots & \ddots & \vdots \\ \sigma^2\rho^n & \sigma^2\rho^{n-1} & \cdots & \sigma^2 \end{pmatrix} \quad (2)$$

This approach is in contrast to standard repeated measures ANOVA, which assumes that the correlation between any two measurements is constant. In addition, use of a GLM accounts for unbalanced designs, in which the number of subjects per group is not equal (an unbalanced design may violate the assumption of orthogonal interaction effects when using a repeated measure ANOVA). All estimation was performed by the linear mixed-effects procedure built into R. In cases where we used a GLM, we represented each participant's response as a single point per block, typically by using the mean value of the outcome variable for each participant within a block. Estimates of the unknown parameters were found using maximum likelihood. We report the adjusted type III error in all cases, which accounts for an unequal number of observations between groups.

When possible, we included the data from Experiment 2 (M1 stimulation) in the statistical tests for Experiment 1 (cerebellar stimulation). Using a single GLM to test for the effect of tDCS across the cerebellum and cortex reduced the total number of statistical tests, thereby reducing spurious multiple comparison effects.

In cases where we found a significant main effect of stimulation, or stimulation by block interaction, we performed post hoc tests on the simple effect of stimulation to determine which groups were significantly different from sham. To guard against false positives that can arise from multiple comparisons, we used Dunnett's t-test for this post hoc comparison. Dunnett's t-test is a multiple comparison corrected approach that is used when a single control group (the sham group) is compared to other groups. All figures show mean ± SEM, unless otherwise specified.

Results

In our experiment, short blocks of field trials alternated with short blocks of error-clamp trials (Fig. 1A). The two day experiment enabled us to measure three separate components of learning: 1) in field trials of Day 1 we assayed error-dependent learning by quantifying how the motor output improved from one trial to the next, 2) in error-clamp trials of Day 1 we assayed the stability of the developing memory by quantifying how the motor output decayed within blocks in the absence of error, and 3) in error-clamp trials of Day 2 we assayed how much of the acquired memory was retained over a 24 hour period. Our principal question was with regard to effects of stimulation of the cerebellum and the motor cortex on these three components.

Our study included four stimulation groups: sham, anodal cerebellar, cathodal cerebellar and anodal M1. Because the same protocol was used for all groups, a single statistical model (GLM) assessed effects across all stimulation groups. However, for clarity of presentation we first report the effects of cerebellar stimulation on a particular set of variables, and then present effects of M1 stimulation on the same set of variables. After completion of the adaptation blocks on Day 1, subjects ranked their level of attention, fatigue, and perceived head discomfort using a visual scale. Self-reported ratings of attention, fatigue, and perceived pain did not differ with stimulation (all $p > .05$).

Effects of tDCS in the null field

To test whether brain stimulation affected basic characteristics of movement such as reaction time and peak velocity, we compared performance in a null field condition in which there was no stimulation (block n1, last 50 trials), to a null field condition with stimulation (block n2). We analyzed peak velocity of the reaching movements for each group (sham, anodal cerebellar, cathodal cerebellar, and anodal M1) and found there was no effect of stimulation type ($F(3,46) = 0.29$, $p > 0.8$) nor a tDCS by block interaction ($F(3,46) = 2.4$, $p > 0.05$). We considered other kinematic measures such as perpendicular displacement or velocity at various times into the movement (100 ms and 200 ms) and found no significant effect of stimulation type, nor any interaction.

Effects of tDCS on reaction time

We quantified reaction times during the null field and force field parts of the experiment. To check whether the stimulation itself produced a change in reaction times, we compared reaction times before stimulation (block n1) to reaction times during stimulation (block n2). We performed a GLM with factors of group, block, and a group x block interaction. We found a main effect of group ($F(3,46) = 2.7$; $p < 0.05$), but no group by block interaction. Post hoc tests indicated that the sham group in general reached with slightly shorter reaction times (around 20 ms, the effect reached significance in comparison of sham vs. cerebellar anodal). However this difference in reaction times was not due to the onset of the stimulation, as it was present even before stimulation onset. Therefore, for unknown reasons the sham group reached with slightly shorter reaction times than other groups.

We quantified the reaction time in the early and late phases of training (first 5 and last 5 blocks). In the early phase of training we found that all stimulation groups had longer reaction times than sham (group effect $F(3,46) = 5.86$, $p = 0.0007$; post hoc testing revealed a significant difference between all stimulation groups and sham, $p < 0.001$ in

each case). However, by the late phase of training this difference had disappeared (no group effect $F(3,46) = 1.8$; $p = 0.14$, no effect of block, $F(5,230) = 1.8$; $p = 0.12$, and no group by block interactions, $F(15,230) = 1.2$; $p = 0.30$).

Finally, we checked to see if there was a difference in reaction times between the various groups that received stimulation. We found no effect of stimulation type ($F(2,35) = 0.82$, $p > 0.4$), and no stimulation type by block interaction ($F(18,315) = 0.93$, $p > 0.5$). That is, stimulation modality did not alter reaction time.

In summary, there were no significant differences in reaction times between the various tDCS groups. However, the reaction times throughout the experiment were shorter (by about 20 ms) for the sham group than other groups. This was not because of brain stimulation, as the differences existed even in the first null block in which there was no stimulation. The differences in reaction time between the tDCS and sham groups disappeared by late phase of training (last 5 blocks, a5–a10), during which all groups exhibited comparable reaction times.

Effect of cerebellar stimulation on feedback control

It has been hypothesized that the motor response to error during a movement can act as a teaching signal, driving corrective changes in the motor commands generated in the subsequent movement (Kawato, 1996). To test whether cerebellar stimulation affected the motor response to error during the reach, we assessed the effect of tDCS on hand velocity perpendicular to the direction of target. We focused our analysis on the first 10 trials of block a1, that is, during the earliest period of exposure to the field, before significant learning had occurred.

Hand velocities parallel and perpendicular to the target were computed separately for every trial and every participant, and then averaged across the first 10 trials (shown in Fig. 1B and C). Parallel velocity appeared indistinguishable between the groups (Fig. 1B). Analysis of the peak parallel velocity confirmed that there was no effect of stimulation in the parallel direction (one-way ANOVA, main effect of tDCS, $F(3,46) = 1.9$, $p > 0.1$). Furthermore, the magnitude of the peak perpendicular velocity, which is a proxy for the early motor response to the perturbation, was not affected by stimulation (one-way ANOVA, main effect of tDCS, $F(3,46) = 0.45$, $p > 0.7$). However, a closer examination of the perpendicular velocity trace suggested that the feedback response to the perturbation appeared to be delayed in the cathodal cerebellar group, separating from the other groups approximately 350 ms into the movement (Fig. 1C). To quantify this potential delay in the feedback response, we considered the time at which the perpendicular velocity crossed zero. This quantity represents the time at which participants had compensated for the cumulative effects of the field, hence allowing us to assess the time within a trial when participants in each tDCS condition compensated for the field. For the cathodal cerebellar group this time was later than for the sham and anodal cerebellar groups (one-way ANOVA, main effect of tDCS, $F(3,46) = 3.4$, $p < 0.05$; post-hoc Dunnett's t-test, cathodal versus sham, $p < 0.05$). By contrast, anodal M1 stimulation had no discernible effects on the ability to respond to sensory feedback: the time when the perpendicular velocity crossed zero was not significantly different between anodal cerebellar stimulation, anodal M1 stimulation and the sham group (peak perpendicular velocity: no main effect of tDCS; zero crossing: post-hoc test, M1 vs. sham, $p > 0.1$).

In summary, we found that cathodal cerebellar stimulation impaired the ability of participants to respond to error feedback during the reach. This delay was not due to a general slowness in visual processing, as reaction times were comparable between various groups that received tDCS.

Effect of cerebellar stimulation on learning from error

To quantify learning from error, we focused on reach kinematics in field trials. Fig. 2A shows average reach trajectories in representative blocks of the experiment for the cerebellar tDCS groups. In the null block (n2) the trajectories appeared indistinguishable. When the perturbation was introduced (block a1), the hand was displaced from its nominal trajectory, and with training the trajectories converged to an "S" shaped path that over-compensated for the perturbation early in the movement and under-compensated late in the movement. In healthy individuals, over-compensation is a characteristic of learning in curl force fields (Izawa et al., 2008; Thoroughman and Shadmehr, 2000). However, this characteristic of force field learning is reduced or missing in people with cerebellar damage (Criscimagna-Hemminger et al., 2010). Here, we found that anodal cerebellar stimulation enhanced over-compensation, whereas cathodal stimulation reduced it (Fig. 2A). This is further illustrated in the perpendicular velocity traces, as shown in Fig. 2B. In the early adaptation block (a1), at the onset of the movement the perpendicular velocity was in the positive direction, reflecting the effect of the perturbation. However, with further training (blocks a6 and a10) the perpendicular velocity progressively shifted in the negative direction, reflecting over-compensation. Over-compensation was evident by block a6 in the anodal condition, but appeared to develop more slowly in the cathodal condition.

To quantify these patterns, we focused on a measure early in the movement, hand velocity perpendicular to the direction of the target at 100 ms after reach onset, and a measure relatively late in the movement, maximum perpendicular displacement. Analysis of other trajectory measures (e.g. perpendicular displacement at 50 ms or 200 ms) confirmed the same pattern of results.

We first considered a measure early in the movement (100 ms) (Fig. 2C). In block a1, the groups showed comparable performance. There was no significant difference between the mean perpendicular velocity at 100 ms in block a1 (one-way ANOVA, main-effect of tDCS, $F(3,46) = 0.2$, $p > 0.8$). However, as training progressed, performance of the three groups diverged. In particular, over-compensation emerged fastest in the anodal group and slowest in the cathodal group (when the data values fall below zero, the motor commands exhibited over-compensation). A GLM with factors of block (a1 to a11) and tDCS found a main effect of stimulation type ($F(3,46) = 2.8$, $p < 0.04$) and block ($F(10,460) = 23.1$, $p < 10^{-3}$). Post hoc comparisons indicated that the cathodal cerebellar group exhibited slower learning, resulting in an increase in the overall perpendicular velocity compared to the sham group (Dunnett's t-test, $p < 0.01$). In contrast, the anodal group showed faster learning compared to sham (Dunnett's t-test, $p < 0.05$).

We next considered a measure that focused on the late part of the movement (peak displacement from a straight line). Fig. 2D plots maximum displacement caused by the perturbation, averaged for each block. The maximum displacement curves of the cerebellar anodal and sham groups appeared indistinguishable, whereas the cathodal group exhibited larger maximum displacement, indicating reduced compensation for the force field. GLM analysis identified a significant main effect of tDCS ($F(3,46) = 2.6$, $p = 0.05$) and block ($F(10,460) = 28.9$, $p < 10^{-3}$), and post-hoc analysis confirmed that the cathodal group exhibited significantly larger maximum displacement than the sham group (Dunnett's t-test, $p < 0.001$) whereas the anodal group showed no significant difference compared to sham (Dunnett's t-test, $p > 0.9$). This reduced compensation in the cathodal group relative to sham may be due to stimulation-induced impairments in learning, or due to impairment of the feedback response reported earlier.

In summary, kinematic measures during training illustrated that anodal cerebellar stimulation increased the learning rate, whereas cathodal stimulation reduced this rate.

Robustness of statistical results

We had $n = 9$ subjects in the cerebellar cathodal group and a larger number of subjects in the sham and cerebellar anodal groups. To what extent could this imbalance in the study population size have affected

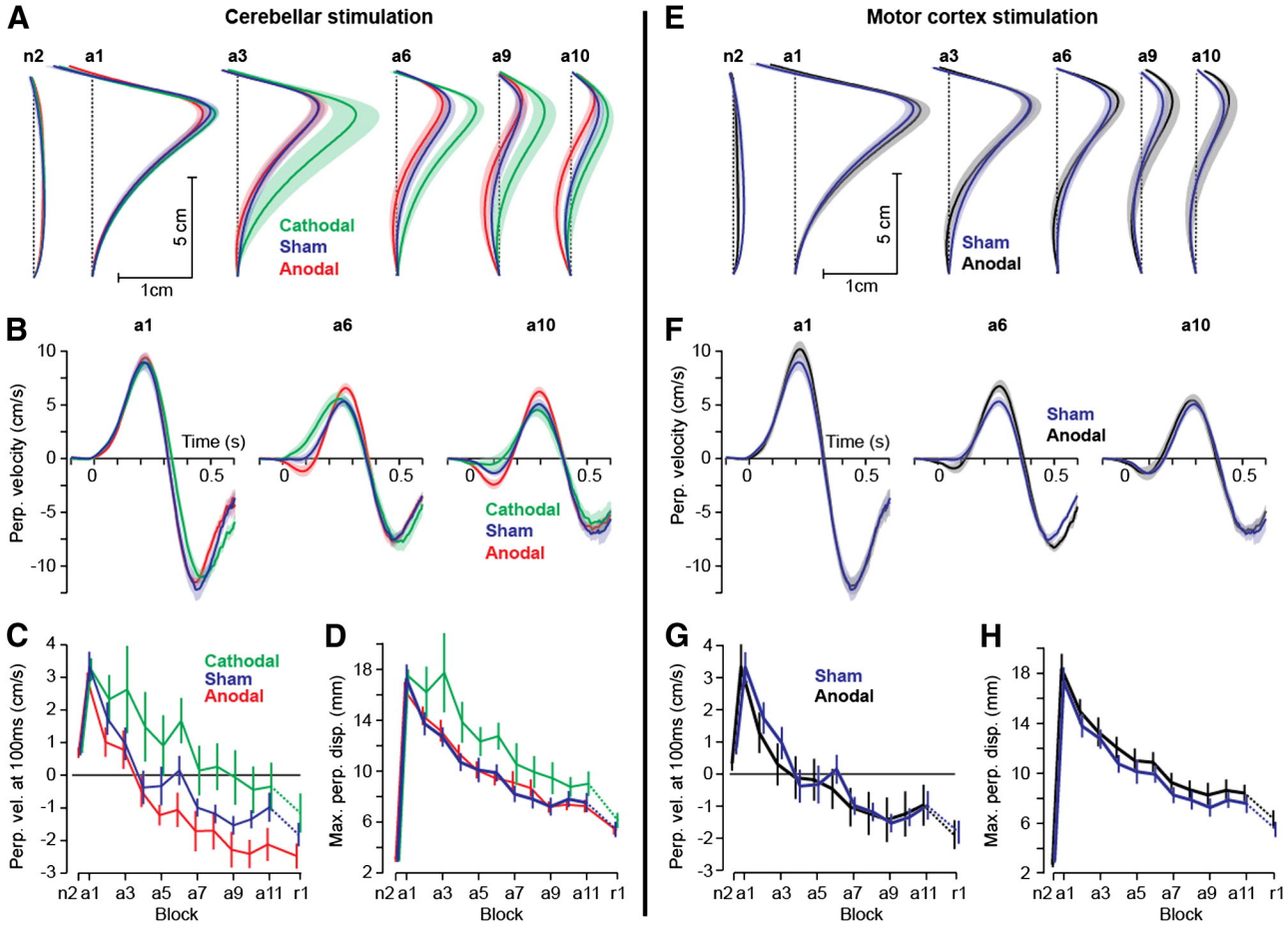

Fig. 2. Reach kinematics and measures of error-dependent learning during cerebellar (left column) and motor cortex (right column) stimulation. A. Hand paths during cerebellar stimulation. The reach starts at the bottom and ends at the top. Figures show across participants mean ± SEM of hand position for each tDCS group during blocks of training labeled at top of the figure. With training, both the anodal and sham tDCS groups exhibited "over-compensation" early in the reach; this effect appeared larger in the anodal group and smaller in the cathodal group. B. Hand velocity (cm/s) perpendicular to the direction of target. Over-compensation gradually emerges across blocks, as reflected in the negative hand velocities in the early period after movement onset (0–100 ms). C. Perpendicular velocity at 100 ms after reach onset during selected blocks of training. Positive values represent clockwise deviation of the hand, and negative values represent over-compensation. D. Maximum displacement of the hand perpendicular to the direction of the target. E–H. The same as parts A–D, but for anodal stimulation of the motor cortex.

our conclusion regarding impairment of learning in the cerebellar cathodal group?

In both GLM and standard one-way ANOVA, the smaller number of subjects in a group increases the estimate of the between-subject variability. Therefore, the cathodal group is at a statistical disadvantage in terms of the likelihood of finding significant results when it is compared to other groups. Despite this, we found that the altered rate of learning during cathodal cerebellar stimulation was the strongest effect in the dataset, showing the highest levels of significance (even compared to anodal cerebellar ($n = 15$) versus sham ($n = 12$)).

A reasonable way to deal with unequal sample size is to use an autoregressive structure of the GLM in which each group has a different variance value (diagonal elements on the variance-covariance matrix). This ensures that when performing post-hoc contrasts, smaller groups, with measured higher variance values, are at a statistical disadvantage. Despite using this conservative approach, we found consistent effects of cerebellar cathodal stimulation.

To directly test the robustness of our inference we performed a bootstrap analysis in which we randomly sampled $n = 9$ subjects from the sham group (without replacement). For each of 100 iterations, we performed a GLM and a post-hoc comparison between the resampled sham ($n = 9$) and cerebellar cathodal groups ($n = 9$) using the metric of perpendicular displacement at 100 ms. We found that the mean p-value for this corrected comparison was 0.013 ± 0.018 (mean ± SD), indicating that the cathodal group learned significantly slower than sham, even when the group sizes were equalized.

Effects of motor cortex stimulation on learning from error

Were the changes in rates of learning specific to the stimulation of the cerebellum? Fig. 2E and F display reach trajectories and perpendicular velocities for anodal M1 and sham groups during various stages of training. Anodal M1 stimulation did not appear to induce significant changes in reach kinematics. For example, the over-compensation early in the reach and the "S" shape of the hand path appeared unaffected by M1 stimulation. These patterns were quantified via perpendicular velocity at 100 ms (Fig. 2G) and maximum perpendicular velocity (Fig. 2H). Following a GLM analysis (reported in the cerebellar section above), a post-hoc comparison did not find a significant difference between M1 and sham groups (Dunnett's t-test, 100 ms: $p > 0.9$, max displacement: $p > 0.1$).

In summary, we observed an increased rate of learning when the cerebellum received anodal stimulation, but not when anodal stimulation was applied to the motor cortex.

Effects of cerebellar stimulation on stability of the motor memory

To assess stability of the acquired motor memory, we focused on the force patterns that the participants produced in error-clamp trials.

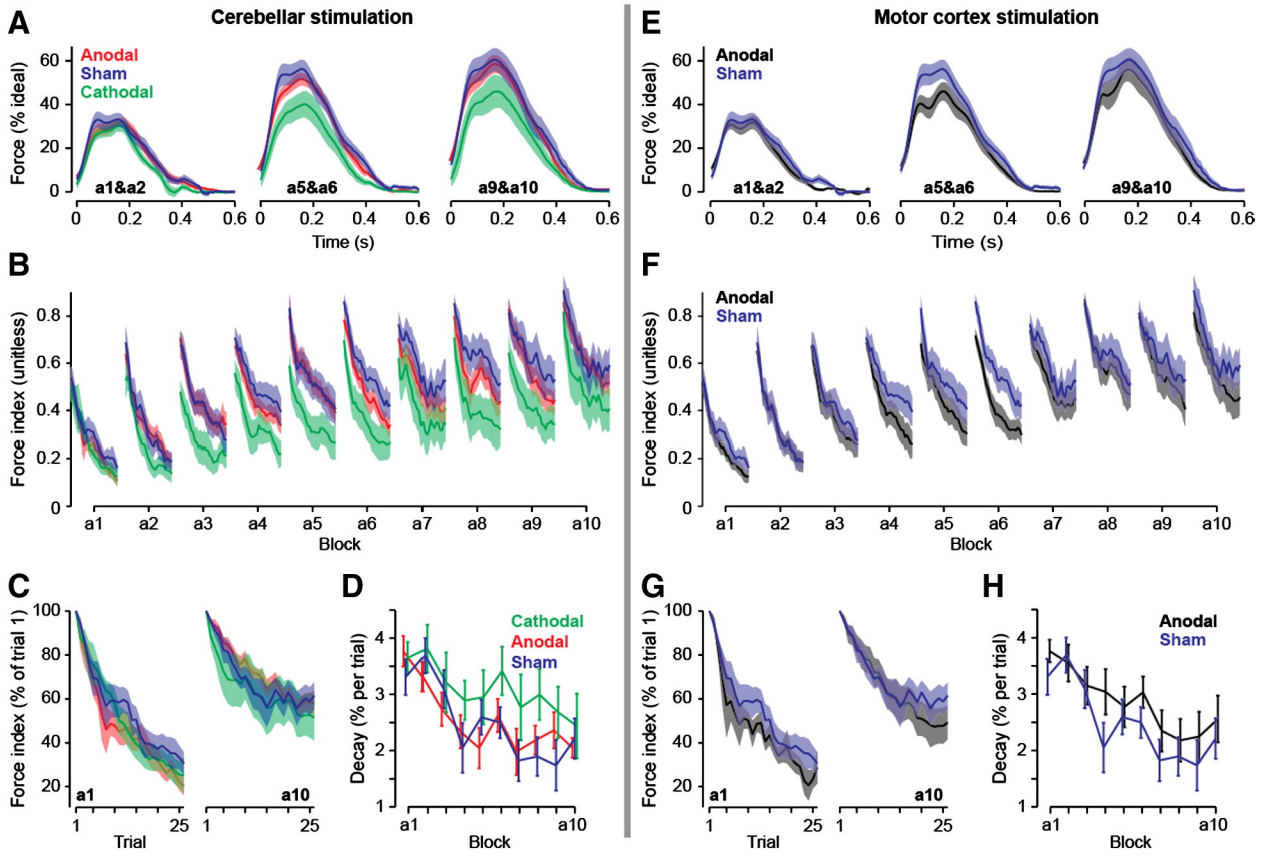

Fig. 3. Force in error-clamp blocks and measures of decay in motor output during cerebellar or motor cortex stimulation. A. Force in error-clamp trials in various blocks as a percentage of ideal force in the cerebellar stimulation group. The ideal force was computed at time of peak velocity during the reach by multiplying velocity by field strength. B. Force index (Eq. (1), a unitless variable) as computed in error-clamp blocks. Data were smoothed using a sliding window with a bin width of 5 trials. C. In error-clamp blocks forces decay. However, with training the memory becomes resistant to decay. Forces were normalized to the first trial of the error-clamp block. The traces represent data from blocks a1 and a10. Data were smoothed using a sliding window with a bin width of 5 trials. D. Decay per trial in each block was estimated by fitting a line to the data shown in part B. The slope of the regression line represents the rate of change in units of % force index per trial. E–H. The same as parts A–D but for anodal stimulation of the motor cortex. Data are mean ± SEM.

Fig. 3A shows examples of these forces. In general, cerebellar stimulation did not alter the shape of the force profiles. Rather, participants who received cathodal cerebellar stimulation tended to produce smaller forces.

We quantified force traces in error-clamp trials by comparing them to the ideal force, as defined in Eq. (1), and computed a force index α (reflecting the fraction of compensation). This measure is shown in Fig. 3B. Two features stand out: 1) block after block, the force index increases, compensating for a greater amount of the perturbation, and 2) within each error-clamp block the force index decreases, reflecting decay of motor output and de-instantiation of the motor memory in the absence of error (Vaswani and Shadmehr, 2013).

To quantify the between-block change in the force index, independent of the within-block decay, we focused on the first five trials of each block. For each participant we computed the average force index across these five trials in each block. A GLM revealed a significant main effect of block ($F(9,414) = 10.0$, $p < 10^{-3}$) as well as a significant effect of tDCS ($F(3,46) = 6.2$, $p < 0.001$). A post-hoc test revealed that the cathodal cerebellar group produced significantly smaller forces in error-clamp trials compared to sham (Dunnett's t-test, $p < 0.001$) (Fig. 3B).

Reach kinematics had shown that anodal stimulation of the cerebellum led to a larger amount of over-compensation than cathodal or sham stimulation (Fig. 2C). However, the force measurements in error-clamp trials did not suggest a difference between the anodal and the sham groups (Dunnett's t-test, $p > 0.4$, and as shown in Fig. 3B). We hypothesized that the reason for this may be that our force measure (force index) had quantified the entire trajectory, rather than focusing on the early component of the movement (when over-compensation occurs). Therefore, we performed further analysis of the force, but now focused on movement onset. We defined movement onset as the time when the reach exceeded 10% of maximum velocity in the direction of the target. This corresponds to time zero on the force traces shown in Fig. 3A. Focusing on force at movement onset, and for the first five trials of each error-clamp block, GLM showed a significant main effect of block ($F(9,414) = 3.2$, $p < 10^{-3}$) and a significant main effect of stimulation type ($F(3,46) = 5.4$, $p < 0.01$). Post-hoc tests revealed that the anodal group produced significantly larger forces compared to sham (Dunnett's one-sided test, $p < 0.05$) but the cathodal group continued to produce significantly smaller forces than sham (Dunnett's one-sided test, $p < 0.001$). In summary, force measurements early in the reach at start of error-clamp blocks confirmed kinematic measurements in field trials, demonstrating increased learning with anodal cerebellar stimulation and decreased learning with cathodal cerebellar stimulation.

A critical question was whether cerebellar stimulation affected the force decay patterns in error-clamp trials. To assess the within error-clamp block change in the force index, we computed the rate at which this index decayed. For example, in a1 the force index was around 0.55 at the start of the error-clamp block (Fig. 3B). This implies that in block a1, in the field trials that had preceded the start of the error-clamp block, participants learned about 55% of the ideal force. As the error-clamp block in a1 ended the forces had decayed to approximately 20%. Therefore, the small number of field trials in a1 produced a great deal of learning (55% of force was learned), but the resulting memory exhibited decay in the absence of error (loss of around 63%). In a10 the force index was around 0.85 at start of the error-clamp block and

decayed to around 0.55 by the end of the block, exhibiting about 35% loss. A useful way to visualize these patterns is to normalize the force measure with respect to the first trial of each error-clamp block, illustrated in Fig. 3C. At the start of training the memory could be described as "fast," exhibiting rapid decay (Smith et al., 2006). With training, the memory decayed less in the error-clamp block, becoming "slow."

To quantify the decay patterns we fitted the data in Fig. 3B to a single line for each block and each participant and measured the slope of that line. The results are shown in Fig. 3D, represented as percent decay per trial. GLM analysis revealed a significant main effect of block ($F(9,411) = 6.9$, $p < 0.001$), but no significant effect of tDCS ($F(3,46) = 1.6$, $p > 0.1$), and no interactions ($F(27,411) = 0.8$, $p > 0.7$). Therefore, cerebellar stimulation did not significantly alter the rate of decay in error-clamp blocks. Similar results were obtained with other measures of performance, such as force at peak velocity.

In summary, analysis of forces in error-clamp trials demonstrated that cathodal cerebellar stimulation slowed the rate at which the brain learned to predict and compensate for the perturbation. In blocks of error-clamp trials, these forces decayed. Early in training the decay per trial was large, but with further training decay per trial became smaller, suggesting that with training the memory gained stability. Cerebellar stimulation did not significantly alter these decay patterns. Therefore, cerebellar stimulation affected the rate of learning, but not the rate of decay of the resulting memory as assayed in error-clamp trials.

Effects of motor cortex stimulation on stability of the motor memory

Fig. 3E displays the force traces produced by the group that received anodal stimulation of the motor cortex, and Fig. 3F summarizes these results using the force index. To quantify the between-block change in the force index, independent of the within-block decay, we focused on the first five trials of each block. For each participant we computed the average force index across these five trials in each block. As reported in the above results, a GLM had revealed a significant effect of block, and significant effect of tDCS. However, a post-hoc test revealed no significant effects of anodal M1 stimulation (Dunnett's t-test, $p > 0.1$).

Fig. 3G and H display the decay properties of the force index, demonstrating that with training the decay rates were reduced (statistics reported above). Nevertheless, anodal M1 stimulation did not produce any significant changes in these decay patterns (as demonstrated by the lack of stimulation effect in the GLM, reported above). Therefore, in contrast to what was observed in a visuomotor rotation experiment (Galea et al., 2011), in this force field task anodal M1 stimulation did not alter the rate of decay of the motor memory in the absence of error.

Effect of stimulation on generalization

After completion of block a11, participants were tested in a generalization block (g2), to assess transfer of performance from trained targets to nearby untrained targets. Reaches to the generalization targets were in error-clamp. A fraction of the reaches to the trained targets were in field trials (to prevent decay of the motor output), and the remaining reaches were in error-clamp trials (to assess the force index). The force index for the generalization trials was expressed as a fraction of the average index for the two trained targets (Fig. 4A). Generalization was tested using a compound-symmetric GLM with factors of direction and tDCS group. There was a significant effect of direction ($F(6,272) = 10.5$, $p < 0.001$), reflecting transfer of training to untrained target locations, but no effect of tDCS ($F(3,46) = 0.3$, $p > 0.8$) and no interaction effect ($F(18,272) = 1.2$, $p > 0.2$). Therefore, stimulation of the motor cortex or the cerebellum did not significantly alter generalization patterns.

Effect of stimulation on over-night retention

Training on Day 1 ended with a final block of field trials (r1), which significantly improved performance with respect to block a11 (Fig. 2D,

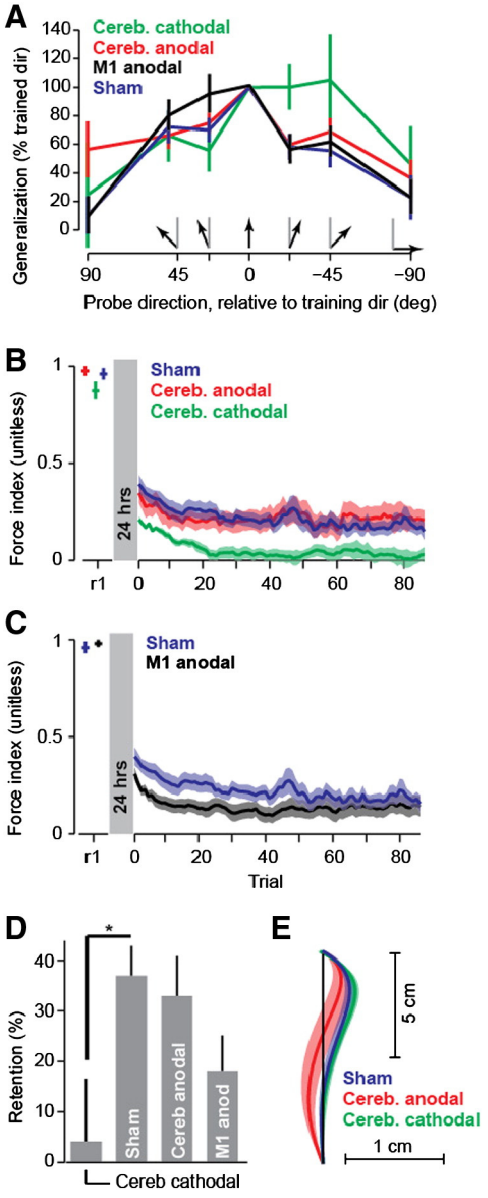

Fig. 4. Generalization and retention. A. Generalization was assayed at end of training in Day 1 (block g2). Force index for each participant at the various probe targets was normalized to each participant's own force index in the trained targets (direction 0). The arrow indicates direction of probe target and the gray line indicates direction of trained target. Data are mean ± SEM. B. Retention at 24 hours following completion of training. Force index at the end of training on Day 1 (block r1), and during testing on Day 2 (block b1) for cerebellar stimulation. The data in block b1 were smoothed with a sliding window using a bin width of 5 trials. C. The same as in part B, but for anodal stimulation of the motor cortex. D. Overnight retention, measured as average force index in block b1 as a percentage of force index in the last 10 trials of block a10. * indicates $p < 0.05$. E. Hand paths in block b2. Data are mean ± SEM.

perpendicular velocity at 100 ms, main effect of block $F(1,46) = 21.9$, $p < 0.001$, with no effects of tDCS, and no interaction). Subsequently, participants left the experiment room and returned a day later. Testing on Day 2 began with a block of error-clamp trials (block b1), which precluded re-exposure to the previously trained force field. As before, we quantified the forces that subjects produced on each error-clamp trial of Day 2 using a force index, and the results are plotted in Fig. 4B and C. A one-way ANOVA on the force index averaged across block b1 revealed a significant effect of stimulation type ($F(3,46) = 3.1$, $p < 0.05$). Post-hoc tests revealed a significant difference between the cathodal and sham groups only (Dunnett's t-test, $p < 0.05$). Therefore, participants who

had received cerebellar cathodal stimulation on Day 1 produced smaller forces on Day 2.

However, learning on Day 1 in the cerebellar cathodal group had been impaired by tDCS. Indeed, the cathodal cerebellar participants did not attain the same level of task performance as the other groups. Hence, between-group differences on Day 2 do not simply reflect differences in retention. To address this issue, we used an approach based on previous work. Joiner and Smith (2008) trained groups of volunteers in a force field task for various durations, yielding different levels of task performance. They then tested each group on Day 2 in error-clamp trials. The authors found that final performance on Day 1 was not a good predictor of forces exerted on Day 2. Rather, a specific component of performance on Day 1 was a good predictor of Day 2: the component attributed to "the slow process," that which in the absence of error shows little decay. The authors showed that, despite different final levels of task performance on Day 1, the amount of force participants produced on Day 2 was a constant fraction of this slow-process component of the forces produced on Day 1.

In our experiment, the error-clamp blocks on Day 1 were 30 trials in duration, long enough to be dominated by the slow process, as the fast process has a time constant in which forces decay by 95% by the 8th trial (Smith et al., 2006). Therefore, to compute retention, we averaged the force index during the final two error-clamp blocks on Day 1 (blocks a9 and a10), and then compared this for each participant to the average force index during the error-clamp block on Day 2 (block b1). The results are plotted in Fig. 4D. ANOVA indicated a significant effect of stimulation type ($F(3,46) = 3.19$, $p < 0.05$), and a post-hoc test revealed impaired retention in the cathodal group (Dunnett's t-test, $p < 0.05$).

Following the error-clamp block b1, re-learning was assessed in a block of field trials (block b2 was examined in a subset of participants from each cerebellar tDCS group, $n = 12/12$ anodal cerebellar, $n = 10/12$ sham, and $n = 8/10$ cathodal). Fig. 4E shows hand trajectories during block b2. All groups exhibited faster re-learning (i.e., "savings"), showing a maximum perpendicular displacement in the first 10 trials of b2 that was within 95% of the final value of block r1 in the previous day. In addition, the anodal group exhibit greater over-compensation than the cathodal group (one-way ANOVA on the perpendicular velocity at 100 ms identified a main effect of stimulation type, $F(2,28) = 3.7$, $p < 0.05$, and Dunnett's post-hoc t-test showed significantly larger over-compensation in the anodal vs. sham groups, $p < 0.05$).

In summary, we found that retention, as measured by the ratio of force produced in error-clamp trials on Day 2 with respect to end of Day 1, was impaired in the cerebellar cathodal group, but unaffected by anodal M1 or anodal cerebellar stimulation.

Discussion

We performed a two day experiment to measure effects of non-invasive brain stimulation on the ability to learn to reach in a force field. We found that increasing the excitability of the cerebellum via anodal tDCS increased the rate of learning, while decreasing cerebellar excitability via cathodal tDCS impaired the ability to respond to sensory feedback and decreased the rate of learning. On Day 1, training resulted in a motor output that decayed in the absence of error. This decay was fast in the early part of training, but with further training the decay slowed, suggesting that with training the motor memory gained stability. Stimulation of the cerebellum or the motor cortex did not alter these decay patterns. On Day 2, when re-exposed to the same learning context, participants reproduced some of the motor commands that they had learned the previous day. Participants who had acquired the task while receiving cathodal cerebellar stimulation exhibited impaired retention, whereas anodal stimulation of the motor cortex or the cerebellum did not alter overnight retention.

Feedback control

When the nervous system detects an error during a reach, motor commands that correct the error and bring the hand to the target originate in the spinal cord, the motor cortex, and the cerebellum. If the cerebellar deep nuclei are cooled, the early component of the error-feedback response (associated with a response in the agonist muscle) is generally unaltered, but the later component (associated with a response in the antagonist muscle) is delayed (Vilis and Hore, 1980). Here, we observed that cathodal cerebellar stimulation reduced the feedback gain of the arm, resulting in motor commands that were slower than normal in correcting for the perturbation. How might cathodal stimulation of the cerebellum affect the error-feedback response? Results from TMS experiments (Ugawa et al., 1995) suggest that cathodal tDCS decreases the resting membrane potential of cerebellar neurons (Galea et al., 2009), apparently decreasing the proportion of cells that respond to input. Because Purkinje cell activity is modulated by unexpected sensory feedback in the context of a self-generated movement (Brooks and Cullen, 2013; Gilbert and Thach, 1977), a reduced sensitivity to mossy fiber input may underlie the impairment in error-feedback response.

Given the extensive evidence regarding the role of the motor cortex in feedback control (Evarts and Tanji, 1976; Kimura et al., 2006), it seems likely that disruption of M1 via cathodal stimulation, something that we did not attempt, would also affect the ability of the brain to respond to a perturbation. An interesting future experiment would be to compare the effects of cathodal stimulation of the cerebellum with M1.

Learning from error

We used reach kinematics to assay learning from error and found that anodal cerebellar stimulation enhanced error-dependent learning, whereas cathodal cerebellar stimulation impaired it. This is consistent with results reported in a visuomotor rotation task, in which anodal cerebellar stimulation enhanced learning (Galea et al., 2011), and in a walking task, where anodal cerebellar stimulation enhanced learning while cathodal stimulation impaired it (Jayaram et al., 2012). Together, the results suggest that the cerebellum is a unique structure that supports the general process of error-dependent motor learning.

In the force field task, the result of learning is not a return to the null, unperturbed trajectory (Izawa et al., 2008). Rather, movements exhibit over-compensation early in the reach and under-compensation late in the reach, resulting in an S-shaped path to the target. Why do the motor commands exhibit over-compensation? The motor commands that are produced in response to the perturbation during the reach may act as a teacher for the brain (Kawato, 1996), driving the change in motor commands that are generated in the subsequent movement (O'Shea et al., 2014). Recordings from muscles show a gradual and orderly transition of the motor commands from one that responds to the perturbation force during the reach (early in training), to one that predicts it near the onset of the reach (late in training) (Thoroughman and Shadmehr, 1999). The fact that cathodal cerebellar stimulation impaired both functions suggests that the later function may benefit from the former.

In contrast to the effects of cerebellar stimulation, we did not observe any effect of anodal stimulation of M1 on learning from error (2 mA, 25 min, 25 cm^2 electrodes). This is consistent with an earlier work in which we found no effect of anodal or cathodal stimulation (1 mA, 20 min, 25 cm^2 electrodes) of M1 in a similar force field task (Orban de Xivry et al., 2011a). Similarly, in a visuomotor rotation task, Galea et al. (2011) used anodal stimulation of M1 (2 mA, 15 min, 25 cm^2 electrodes) and found no effects of stimulation during training in the presence of the perturbation and no differences in the subsequent after-effects when the perturbation was removed. By contrast, Hunter et al. (2009) reported that anodal tDCS of M1 (1 mA, 17 min, 35 cm^2 electrodes) produced a larger reduction in kinematic errors from the

first to the 4th block of force-field training than did sham stimulation (their "signed-error" measure). Following training in the field, these subjects were exposed to a null field condition, in which they exhibited after-effects. However, using the same "signed-error" measure Hunter et al. (2009) did not find an effect of tDCS on the resulting after-effect. Hence, the results of tDCS studies do not, at present, paint a consistent picture of the function of M1 during motor learning. Most of the studies to date, however, have found that learning from error is not affected by anodal stimulation of M1.

Functional stages of motor memory

While there are many factors that can affect kinematic performance in field trials, including changes in muscle co-contraction (Thoroughman and Shadmehr, 1999), and changes in the gain of the long-latency sensory feedback pathways (Ahmadi-Pajouh et al., 2012; Kimura and Gomi, 2009; Kimura et al., 2006), in error-clamp trials these factors are eliminated. Forces that participants produce in error-clamp trials are a proxy for a model that the brain constructs, associating state of the limb to expected perturbation forces (Hwang and Shadmehr, 2005; Sing et al., 2009). In Smith et al. (2006) we predicted that early in training, motor memory was "fast," decaying rapidly in the absence of error, but that with further training, the memory was transformed to "slow," showing gradual decay. Here we found direct evidence for this prediction: we observed that early in training the decay rates of motor output in error-clamp trials were high, but with further training the decay rates declined by about 50% (Fig. 3C). Therefore, with increased practice motor memory gained stability, as reflected in its decay properties in the absence of error. What was the neural basis of this transformation?

In our study, we found no effect of cerebellar or M1 tDCS on the rate of decay of the motor memory. By contrast, Galea et al. (2011) in a visuomotor rotation task found that anodal M1 stimulation reduced the decay rate of the learned motor output (assayed after learning/tDCS had finished, specifically when no visual feedback was provided). In our experiment, we repeatedly measured the decay rate of the evolving motor memory in the absence of error (error-clamp trials). Despite repeated measurements, we found no effects of M1 anodal stimulation. Of course, a null result does not constitute evidence of no effect. Nevertheless, our null effect observations are consistent with another work on force field learning (Orban de Xivry et al., 2011a), in which anodal or cathodal stimulation to M1, or anodal stimulation to the posterior parietal cortex (1 mA, 20 min), did not change the decay rates. What could explain this difference with respect to Galea et al.'s findings?

First, learning in force fields and visuomotor rotations engage distinct areas of the cerebellum (Donchin et al., 2012), and the cerebral cortex (Diedrichsen et al., 2005). This difference in functional anatomy may underlie the reported differences in the effects of M1 stimulation in force field and visuomotor tasks. For example, our earlier work on visuomotor rotation (Hadipour-Niktarash et al., 2007) found that M1 TMS during exposure did not affect the ability to learn from error, but resulted in a motor memory that was fragile, exhibiting rapid decay. Hence, for visuomotor rotation, our earlier results and those of Galea et al. (2011) are consistent, both finding no evidence of a functional role for M1 in learning from error, and both suggesting a role for M1 in the decay of the resulting motor memory. By contrast with these results for visuomotor rotation, in a force field task rTMS of M1 did not induce a deficit in retention, as assayed immediately after learning/stimulation (Baraduc et al., 2004), an effect that appears inconsistent with the predictions of Galea et al. (2011). The existing brain stimulation data suggest that different functional substrates mediate learning in visuomotor rotation and force fields.

Second, in our experimental design we included periodic error-clamp blocks, interleaved amongst blocks of learning in the field. Because the error-clamp blocks induce decay, they may reduce the overall amount of learning achieved during the task, and also reduce the rate of repetition of the motor commands, a natural component of most motor learning paradigms. Repetition is thought to produce a form of memory that is distinct from the memory that is produced from error-dependent learning (Diedrichsen et al., 2010; Huang et al., 2011). Importantly, repetition may produce a memory that depends on the cerebral cortex (Orban de Xivry et al., 2011a). Hence, it is possible that if we had included a greater degree of repetition of motor commands in our training protocol, anodal M1 stimulation may have slowed memory decay in error-clamp trials. Future work could test this hypothesis.

Finally, the way in which decay is assayed may change the effects of anodal M1 stimulation. Here, we measured decay using error-clamp trials in which the proprioceptive and visual error components of each movement were artificially constrained. In contrast, Galea et al. (2011) measured decay in trials in which visual feedback was withheld. Continuous feedback versus no-feedback trials have been shown to elicit differences in the rate of adaptation, and by extension, this difference likely impacts on the decay of acquired motor memories (Kitago et al., 2013). In particular, Galea et al. (2011) showed that when subjects were exposed to washout after learning, using a full-visual feedback condition, which requires a combination of learning from error as well as extinction of the acquired memories, there was no difference in the rate of decay with M1 anodal tDCS versus sham.

In summary, whereas our study of force field learning found that anodal M1 stimulation did not change the decay properties of the motor memory during acquisition, as assayed using error-clamp trials, the same stimulation in visuomotor rotation has been reported to reduce the decay rate of the acquired motor memory measured after learning. Hence, in combination, brain stimulation evidence to date suggests a role for M1 in stabilizing the motor memory that results from visuomotor rotation but not force field learning.

Retention

When participants returned on Day 2, they held the robot handle and reached in error-clamp trials. They produced forces that were correlated with those that they had learned on Day 1, demonstrating retention. These forces were significantly smaller in the cathodal cerebellar group than other groups. However, the critical question was whether this effect was a reflection of the fact that they had learned to a lesser degree on Day 1, or whether the performance on Day 2 was evidence for reduced retention over and beyond the basic effect associated with acquisition. Therefore, to measure retention, we faced the issue that learning had been impaired in Day 1 in the cerebellar cathodal group: they had not reached the same levels of performance as other groups. To solve this problem, we used the analytic approach developed by Joiner and Smith (2008), which showed that retention of force field learning on Day 2, as assayed in error-clamp trials, was a constant fraction of the slow-component of forces produced on Day 1. We found that cathodal cerebellar stimulation showed significantly impaired retention. Anodal cerebellar or M1 stimulation had no effect on overnight retention.

Our results on the potential role of the cerebellum in retention are intriguing because of other results from the force field learning literature. Imaging studies of the cerebellum in the force field task suggest that during multi-week training activity in the anterior cerebellar cortex decreases while activity in the deep nuclei increases (Nezafat et al., 2001). In other motor tasks (e.g. VOR or optokinetic reflex), there is also evidence for this interplay between the cerebellar cortex and nuclei during acquisition and retention (Kassardjian et al., 2005; Okamoto et al., 2011a; Okamoto et al., 2011b).

The fact that we did not observe an effect of anodal M1 stimulation contrasts with the results of Reis et al. (2009), who examined a skill learning task and found that M1 anodal stimulation produced greater over-night learning gains than sham. This highlights a potential difference between error-dependent learning, which appears to rely predominantly on the cerebellum, and skill learning, which has been proposed to rely more on the cerebral cortex.

Generalization

Generalization can be viewed as a signature of the tuning properties of the cells that participate in learning (Shadmehr, 2004). Force field learning produces narrow generalization to neighboring directions of movements, and broad generalization to neighboring positions of the arm, consistent with a neural coding that relies on proprioception (Hwang and Shadmehr, 2005). We have previously found that stimulation of M1 altered spatial generalization patterns, producing greater generalization in joint coordinates of the arm (Orban de Xivry et al., 2011a). Here, we found that stimulation of the cerebellum or M1 did not affect directional generalization patterns, i.e., learning declined as a function of distance to the trained target. An important future experiment is to compare the effects of cerebellar and M1 stimulation on spatial generalization.

Limitations

Given the size of the tDCS electrodes (25 cm^2), and the dipole nature of a direct current stimulation montage, it seems likely that stimulation was not confined solely to the cerebellum or M1. For instance, it is well-established that M1 tDCS alters the excitability of the motor corticospinal tract (Nitsche and Paulus, 2000), and also changes functional brain activity in distal inter-connected brain regions, with the pattern of spread varying with cognitive state (Lang et al., 2005; Stagg et al., 2009). The functional consequence of these distal changes is unclear. With cerebellar stimulation, physiological evidence (MEPs) indicates that the tDCS-induced changes in measures of cerebellar-brain inhibition do not arise from local spread of current to the adjacent brainstem or visual cortex (Galea et al., 2009, 2011). Nevertheless, It is possible that cerebellar tDCS affects processing in M1 and thalamus by changing tonic neural activity in the cerebello–thalamo–cortical pathway. Hamada et al. (2012) aimed to test this physiologically, by assessing sensory evoked potentials in M1 before and after anodal cerebellar tDCS, but they found no change in the excitability of these pathways. Hence, while future work is required to characterize the spatial distribution of tDCS-induced changes in functional brain activity, the available evidence, though not conclusive, does suggest that the current induced by the stimulation protocols used here probably affected mainly the cerebellum or the motor cortex.

We measured feedback response during the early phase of learning, and not in a situation where the perturbations were random. This potentially confounds the ability to learn from error (trial-to-trial change in motor commands), with the ability to correct for error (within trial change in motor commands). However, we think that we can dissociate these two factors: the main effect of learning from error was to produce changes very early in the movement, reflected in the perpendicular displacement at near movement onset (Fig. 2B), whereas the stimulation induced differences that we attributed to feedback control occurred late in the movement (Fig. 1B). Regardless, we envision a future experiment that includes continuous measurements of muscle activity in the context of feedback responses during cerebellar or M1 stimulation.

We found that anodal M1 stimulation produced no significant enhancement of learning or retention. Given the substantial neurophysiological evidence for involvement of M1 in the force field task (Arce et al., 2010a; Li et al., 2001), and the fact that rTMS of M1 impairs overnight retention (Richardson et al., 2006) and the ability to switch from learning of one field to another (Cothros et al., 2006), an important next experiment is to compare the effects of cathodal M1 stimulation with cathodal cerebellar stimulation.

Summary

In summary, we demonstrated that anodal stimulation of the cerebellum enhanced the error-dependent learning process, whereas cathodal stimulation impaired it. We demonstrated that with training, the motor memory was transformed from a process that decayed rapidly in the absence of error, to one that decayed slowly. Neither cerebellar nor motor cortical stimulation affected this transformation. Finally, we found that cathodal stimulation of the cerebellum during acquisition resulted in impaired retention as measured in 24 hours. Overall, we found a critical role for the human cerebellum in the ability to correct for error during a movement, the ability to learn from that error, and the ability to retain the resulting motor memory.

Acknowledgments

This work was supported by grants from the NIH (NS37422) and the Human Frontiers Science Program. A.M.H. is currently with the Department of Neurology, Johns Hopkins School of Medicine. D.P. was supported by a Rotary International Ambassadorial Scholarship. J.O'S. was supported by a Royal Society Dorothy Hodgkin Fellowship and Research Grant.

Conflict of interest

The authors declare no competing financial interests.

References

Ahmadi-Pajouh, M.A., Towhidkhah, F., Shadmehr, R., 2012. Preparing to reach: selecting an adaptive long-latency feedback controller. J. Neurosci. 32, 9537–9545.
Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control AC-19, 716–723.
Arce, F., Novick, I., Mandelblat-Cerf, Y., Israel, Z., Ghez, C., Vaadia, E., 2010a. Combined adaptiveness of specific motor cortical ensembles underlies learning. J. Neurosci. 30, 5415–5425.
Arce, F., Novick, I., Mandelblat-Cerf, Y., Vaadia, E., 2010b. Neuronal correlates of memory formation in motor cortex after adaptation to force field. J. Neurosci. 30, 9189–9198.
Baraduc, P., Lang, N., Rothwell, J.C., Wolpert, D.M., 2004. Consolidation of dynamic motor learning is not disrupted by rTMS of primary motor cortex. Curr. Biol. 14, 252–256.
Block, H., Celnik, P., 2013. Stimulating the cerebellum affects visuomotor adaptation but not intermanual transfer of learning. Cerebellum 12, 781–793.
Brooks, J.X., Cullen, K.E., 2013. The primate cerebellum selectively encodes unexpected self-motion. Curr. Biol. 23, 947–955.
Chen, H., Hua, S.E., Smith, M.A., Lenz, F.A., Shadmehr, R., 2006. Effects of human cerebellar thalamus disruption on adaptive control of reaching. Cereb. Cortex 16, 1462–1473.
Cothros, N., Kohler, S., Dickie, E.W., Mirsattari, S.M., Gribble, P.L., 2006. Proactive interference as a result of persisting neural representations of previously learned motor skills in primary motor cortex. J. Cogn. Neurosci. 18, 2167–2176.
Criscimagna-Hemminger, S.E., Shadmehr, R., 2008. Consolidation patterns of human motor memory. J. Neurosci. 28, 9610–9618.
Criscimagna-Hemminger, S.E., Bastian, A.J., Shadmehr, R., 2010. Size of error affects cerebellar contributions to motor learning. J. Neurophysiol. 103, 2275–2284.
Diedrichsen, J., Hashambhoy, Y., Rane, T., Shadmehr, R., 2005. Neural correlates of reach errors. J. Neurosci. 25, 9919–9931.
Diedrichsen, J., White, O., Newman, D., Lally, N., 2010. Use-dependent and error-based learning of motor behaviors. J. Neurosci. 30, 5159–5166.
Donchin, O., Rabe, K., Diedrichsen, J., Lally, N., Schoch, B., Gizewski, E.R., Timmann, D., 2012. Cerebellar regions involved in adaptation to force field and visuomotor perturbation. J. Neurophysiol. 107, 134–147.
Evarts, E.V., Tanji, J., 1976. Reflex and intended responses in motor cortex pyramidal tract neurons of monkey. J. Neurophysiol. 39, 1069–1080.
Ferrucci, R., Marceglia, S., Vergari, M., Cogiamanian, F., Mrakic-Sposta, S., Mameli, F., Zago, S., Barbieri, S., Priori, A., 2008. Cerebellar transcranial direct current stimulation impairs the practice-dependent proficiency increase in working memory. J. Cogn. Neurosci. 20, 1687–1697.
Galea, J.M., Jayaram, G., Ajagbe, L., Celnik, P., 2009. Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. J. Neurosci. 29, 9115–9122.
Galea, J.M., Sami, S.A., Albert, N.B., Miall, R.C., 2010. Secondary tasks impair adaptation to step- and gradual-visual displacements. Exp. Brain Res. 202, 473–484.
Galea, J.M., Vazquez, A., Pasricha, N., de Xivry, J.J.O., Celnik, P., 2011. Dissociating the roles of the cerebellum and motor cortex during adaptive learning: the motor cortex retains what the cerebellum learns. Cereb. Cortex 21, 1761–1770.
Gandiga, P.C., Hummel, F.C., Cohen, L.G., 2006. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clin. Neurophysiol. 117, 845–850.
Gilbert, P.F.C., Thach, W.T., 1977. Purkinje cell activity during motor learning. Brain Res. 128, 309–328.
Hadipour-Niktarash, A., Lee, C.K., Desmond, J.E., Shadmehr, R., 2007. Impairment of retention but not acquisition of a visuomotor skill through time-dependent disruption of primary motor cortex. J. Neurosci. 27, 13413–13419.
Hamada, M., Strigaro, G., Murase, N., Sadnicka, A., Galea, J.M., Edwards, M.J., Rothwell, J.C., 2012. Cerebellar modulation of human associative plasticity. J. Physiol. 590, 2365–2374.

Huang, V.S., Haith, A., Mazzoni, P., Krakauer, J.W., 2011. Rethinking motor learning and savings in adaptation paradigms: model-free memory for successful actions combines with internal models. Neuron 70, 787–801.
Hunter, T., Sacco, P., Nitsche, M.A., Turner, D.L., 2009. Modulation of internal model formation during force field-induced motor learning by anodal transcranial direct current stimulation of primary motor cortex. J. Physiol. 587, 2949–2961.
Hwang, E.J., Shadmehr, R., 2005. Internal models of limb dynamics and the encoding of limb state. J. Neural Eng. 2, S266–S278.
Izawa, J., Rane, T., Donchin, O., Shadmehr, R., 2008. Motor adaptation as a process of reoptimization. J. Neurosci. 28, 2883–2891.
Jayaram, G., Tang, B., Pallegadda, R., Vasudevan, E.V., Celnik, P., Bastian, A., 2012. Modulating locomotor adaptation with cerebellar stimulation. J. Neurophysiol. 107, 2950–2957.
Joiner, W.M., Smith, M.A., 2008. Long-term retention explained by a model of short-term learning in the adaptive control of reaching. J. Neurophysiol. 100, 2948–2955.
Kaski, D., Quadir, S., Patel, M., Yousif, N., Bronstein, A.M., 2012. Enhanced locomotor adaptation aftereffect in the "broken escalator" phenomenon using anodal tDCS. J. Neurophysiol. 107, 2493–2505.
Kassardjian, C.D., Tan, Y.F., Chung, J.Y., Heskin, R., Peterson, M.J., Broussard, D.M., 2005. The site of a motor memory shifts with consolidation. J. Neurosci. 25, 7979–7985.
Kawato, M., 1996. Learning internal models of the motor apparatus. In: Bloedel, J.R., Ebner, T.J., Wise, S.P. (Eds.), The Acquisition of Motor Behavior in Vertebrates. MIT Press, Cambridge, MA, pp. 409–430.
Kimura, T., Gomi, H., 2009. Temporal development of anticipatory reflex modulation to dynamical interactions during arm movement. J. Neurophysiol. 102, 2220–2231.
Kimura, T., Haggard, P., Gomi, H., 2006. Transcranial magnetic stimulation over sensorimotor cortex disrupts anticipatory reflex gain modulation for skilled action. J. Neurosci. 26, 9272–9281.
Kitago, T., Ryan, S.L., Mazzoni, P., Krakauer, J.W., Haith, A.M., 2013. Unlearning versus savings in visuomotor adaptation: comparing effects of washout, passage of time, and removal of errors on motor memory. Front. Hum. Neurosci. 7, 307.
Kluzik, J., Diedrichsen, J., Shadmehr, R., Bastian, A.J., 2008. Reach adaptation: what determines whether we learn an internal model of the tool or adapt the model of our arm? J. Neurophysiol. 100, 1455–1464.
Laird, N.M., Ware, J.H., 1982. Random-effects models for longitudinal data. Biometrics 38, 963–974.
Lang, N., Siebner, H.R., Ward, N.S., Lee, L., Nitsche, M.A., Paulus, W., Rothwell, J.C., Lemon, R.N., Frackowiak, R.S., 2005. How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? Eur. J. Neurosci. 22, 495–504.
Li, C.S.R., Padoa-Schioppa, C., Bizzi, E., 2001. Neuronal correlates of motor performance and motor learning in the primary motor cortex of monkeys adapting to an external force field. Neuron 30, 593–607.
Nezafat, R., Shadmehr, R., Holcomb, H.H., 2001. Long-term adaptation to dynamics of reaching movements: a PET study. Exp. Brain Res. 140, 66–76.
Nitsche, M.A., Paulus, W., 2000. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J. Physiol. 527 (Pt 3), 633–639.
Okamoto, T., Endo, S., Shirao, T., Nagao, S., 2011a. Role of cerebellar cortical protein synthesis in transfer of memory trace of cerebellum-dependent motor learning. J. Neurosci. 31, 8958–8966.
Okamoto, T., Shirao, T., Shutoh, F., Suzuki, T., Nagao, S., 2011b. Post-training cerebellar cortical activity plays an important role for consolidation of memory of cerebellum-dependent motor learning. Neurosci. Lett. 504, 53–56.
Orban de Xivry, J.J., Criscimagna-Hemminger, S.E., Shadmehr, R., 2011a. Contributions of the motor cortex to the adaptive control of reaching depend on the perturbation schedule. Cereb. Cortex 21, 1475–1484.
Orban de Xivry, J.J., Marko, M.K., Pekny, S.E., Pastor, D., Izawa, J., Celnik, P., Shadmehr, R., 2011b. Stimulation of the human motor cortex alters generalization patterns of motor learning. J. Neurosci. 31, 7102–7110.
Orban de Xivry, J.J., Ahmadi-Pajouh, M.A., Harran, M.D., Salimpour, Y., Shadmehr, R., 2013. Changes in corticospinal excitability during reach adaptation in force fields. J. Neurophysiol. 109, 124–136.
O'Shea, J., Gaveau, V., Kandel, M., Koga, K., Susami, K., Prablanc, C., Rossetti, Y., 2014. Kinematic markers dissociate error correction from sensorimotor realignment during prism adaptation. Neuropsychologia 55, 15–24.
Reis, J., Schambra, H.M., Cohen, L.G., Buch, E.R., Fritsch, B., Zarahn, E., Celnik, P.A., Krakauer, J.W., 2009. Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. Proc. Natl. Acad. Sci. U. S. A. 106, 1590–1595.
Richardson, A.G., Overduin, S.A., Valero-Cabre, A., Padoa-Schioppa, C., Pascual-Leone, A., Bizzi, E., Press, D.Z., 2006. Disruption of primary motor cortex before learning impairs memory of movement dynamics. J. Neurosci. 26, 12466–12470.
Scheidt, R.A., Reinkensmeyer, D.J., Conditt, M.A., Rymer, W.Z., Mussa-Ivaldi, F.A., 2000. Persistence of motor adaptation during constrained, multi-joint, arm movements. J. Neurophysiol. 84, 853–862.
Shadmehr, R., 2004. Generalization as a behavioral window to the neural mechanisms of learning internal models. Hum. Mov. Sci. 23, 543–568.
Shadmehr, R., Brashers-Krug, T., 1997. Functional stages in the formation of human long-term motor memory. J. Neurosci. 17, 409–419.
Shadmehr, R., Mussa-Ivaldi, F.A., 1994. Adaptive representation of dynamics during learning of a motor task. J. Neurosci. 14, 3208–3224.
Shadmehr, R., Brandt, J., Corkin, S., 1998. Time-dependent motor memory processes in amnesic subjects. J. Neurophysiol. 80, 1590–1597.
Sing, G.C., Joiner, W.M., Nanayakkara, T., Brayanov, J.B., Smith, M.A., 2009. Primitives for motor adaptation reflect correlated neural tuning to position and velocity. Neuron 64, 575–589.
Smith, M.A., Shadmehr, R., 2005. Intact ability to learn internal models of arm dynamics in Huntington's disease but not cerebellar degeneration. J. Neurophysiol. 93, 2809–2821.
Smith, M.A., Ghazizadeh, A., Shadmehr, R., 2006. Interacting adaptive processes with different timescales underlie short-term motor learning. PLoS Biol. 4, e179.
Stagg, C.J., O'Shea, J., Kincses, Z.T., Woolrich, M., Matthews, P.M., Johansen-Berg, H., 2009. Modulation of movement-associated cortical activation by transcranial direct current stimulation. Eur. J. Neurosci. 30, 1412–1423.
Thoroughman, K.A., Shadmehr, R., 1999. Electromyographic correlates of learning internal models of reaching movements. J. Neurosci. 19, 8573–8588.
Thoroughman, K.A., Shadmehr, R., 2000. Learning of action through adaptive combination of motor primitives. Nature 407, 742–747.
Ugawa, Y., Uesaka, Y., Terao, Y., Hanajima, R., Kanazawa, I., 1995. Magnetic stimulation over the cerebellum in humans. Ann. Neurol. 37, 703–713.
Vaswani, P.A., Shadmehr, R., 2013. Decay of motor memories in the absence of error. J. Neurosci. 33, 7700–7709.
Vilis, T., Hore, J., 1980. Central neural mechanisms contributing to cerebellar tremor produced by limb perturbations. J. Neurophysiol. 43, 279–291.