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Cerebellum and M1 interaction during early learning of timed motor sequences

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We used positron emission tomography (PET) to examine within-day learning of timed motor sequences. The results of this experiment are novel in showing an interaction between cerebellum and primary motor cortex (M1) during learning that appears to be mediated by the dentate nucleus (DN) and in demonstrating that activity in these regions is directly related to performance. Subjects were scanned during learning (LRN) across three blocks of practice and during isochronous (ISO) and perceptual (PER) baseline conditions. CBF was compared across blocks of learning and between the LRN and baseline conditions. Results demonstrated an interaction between the cerebellum and M1 such that earlier, poorer performance was associated with greater activity in the cerebellar hemispheres and later, better performance was associated with greater activity in M1. Inter-regional correlation analyses confirmed that as CBF in the cerebellum decreases, blood flow in M1 increases. Importantly, these analyses also revealed that activity in cerebellar cortex was positively correlated with activity in right DN and that DN activity was negatively correlated with blood flow in M1. Activity in the cerebellar hemispheres early in learning is likely related to error correction mechanisms which optimize movement kinematics resulting in improved performance. Concurrent DN activity may be related to encoding of this information and DN output to M1 may play a role in consolidation processes that lay down motor memories. Increased activity in M1 later in learning may reflect strengthening of synaptic connections associated with changes in motor maps that are characteristic of learning in both animals and humans.

Keywords: Motor-skill learning; Cerebellum; Dentate nucleus; Motor cortex; Basal ganglia; Human

Introduction

A growing body of evidence in both animals and humans has demonstrated plastic neuronal changes in the brain with learning of a motor skill (Doyon and Ungerleider, 2002; Doyon et al., 1996, 1999, 2002, 2003; Gandolfo et al., 2000; Graybiel, 1995; Hikosaka et al., 2002b; Kleim et al., 2002a; Nudo et al., 1996; Thach, 1996). These experiments can be roughly divided into two categories: those that have focused on early rapid changes occurring over minutes (Classen et al., 1998; Doyon et al., 1996, 1999, 2002; Imamizu et al., 2000; Karni et al., 1995; Nezafat et al., 2001; Pascual-Leone et al., 1994; Shadmehr and Holcomb, 1997; Toni et al., 1998; Van Mier et al., 1998); and those that have examined relatively slowly developing changes occurring over days or weeks (Karni et al., 1995; Kleim et al., 2004; Lu et al., 1998; Nezafat et al., 2001; Nudo et al., 1996; Pascual-Leone et al., 1995; Penhune and Doyon, 2002). The results of these experiments have demonstrated the involvement of specific regions of motor cortex, the cerebellum and basal ganglia (BG) depending of the stage of motor learning. Drawing on work in experimental animals, Kleim et al. (2002a, 2004) has hypothesized that early rapid plasticity of motor maps in M1 may be mediated by unmasking of latent connections, while longer-term changes are mediated by synaptogenesis and strengthening of cortical connections (Rioult-Pedotti et al., 1998). In the cerebellum, early learning is probably mediated by error-correction mechanisms instantiated in the climbing fiber system of the cerebellar cortex (Ito, 2000), while later learning may involve plastic changes in regions of the cerebellar hemispheres and/or the cerebellar nuclei specific to the effector and internal model for the task (Imamizu et al., 2000; Lu et al., 1998; Nezafat et al., 2001). In the BG, it has been proposed that anterior putamen is more involved in early learning, while the posterior region is more important for later learning (Jueptner and Weiller, 1998; Miyachi et al., 2002).

More recently, it has been proposed that distinct cortico-cerebellar and cortico-striatal systems may be important for different stages of learning (Doyon and Ungerleider, 2002; Doyon

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et al., 2003), different modalities of learning (Doya, 2000, 2003) or for learning different aspects of the same task (Hikosaka et al., 2002a; Middleton and Strick, 2000). Strikingly, however, there is little data allowing comparison of the neural mechanisms underlying the early and late periods of learning on the same task (Karni et al., 1995; Kleim et al., 2004; Nezafat et al., 2001). In a previous study of across-day learning (Penhune and Doyon, 2002), we showed that a dynamic network including the cerebellum, basal ganglia and motor cortical regions were differentially active on Day 1 of practice, after 5 days of training and at delayed recall. Based on these results, we proposed that the cerebellum is critically involved in optimizing movement kinematics during early learning, but that later learning and delayed recall are mediated by the BG and motor cortical regions. Therefore, the present experiment was designed to examine within-day changes in the cortico-cerebellar and cortical–striatal networks. Most importantly, the experiment was designed to allow the direct assessment of the relationship between behavioral measures of learning and changes in the pattern of active brain regions and to allow the examination of the interaction between different brain regions across the course of learning.

Motor sequence learning in this experiment was conceptualized as the optimization with practice of specific parameters of motor response that result in improved precision and accuracy of performance. This is similar to the type of motor learning examined in studies of serial finger tapping (Karni et al., 1995) and force field learning (Nezafat et al., 2001). This contrasts with other paradigms, such as the serial reaction time task (SRT) that emphasize implicit or explicit learning of the order of a sequence of movements. The task used was the timed motor sequence task (TMST) developed in our previous study of across-day learning (Penhune and Doyon, 2002). The TMST requires subjects to reproduce a temporally complex sequence of finger taps in synchrony with a visual stimulus (Fig. 1, panel A). Subjects were scanned across three blocks of learning on the same task along with two baseline conditions. In order to identify changes in the pattern of active regions during learning, subtraction analyses contrasted blood flow across blocks of learning and between the learning and baseline conditions. To confirm the results of the subtraction analyses, normalized blood flow was extracted from regions identified in the subtraction analysis. Most importantly, regression analyses were performed to examine the relationship between behavioral measures of performance and blood flow across blocks of learning. Finally, inter-regional regression analyses were conducted to examine the interaction of the cerebellar and motor cortical regions seen to be active across blocks of learning. The results of this experiment are novel in showing a direct relationship between blood flow and performance, and in showing an interaction between the cerebellum and M1 during learning.

Materials and methods

Subjects

Subjects were 12 healthy, right-handed volunteers selected to have no more than 1 year of musical training or experience (6 female, 6 male, average age = 24.8). Subjects were paid for their participation, and gave informed consent. The experimental protocol was approved by the Research Ethics Committee of the Montreal Neurological Institute.

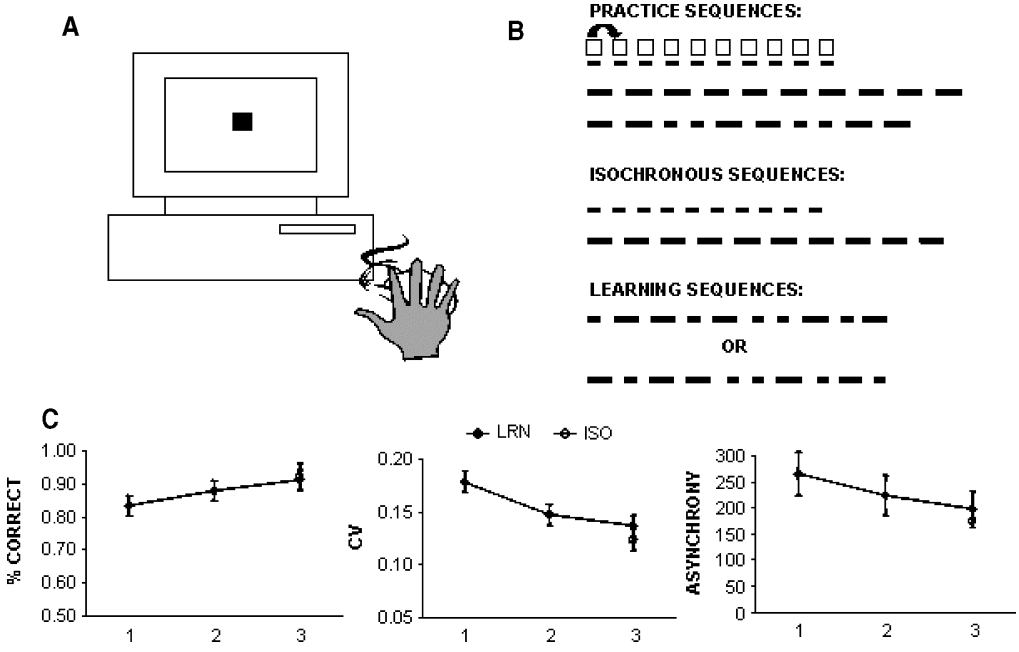

Fig. 1. Illustrates the experimental setup, stimulus sequences and behavioral results. Stimulus sequences were made up of white squares which appeared sequentially at the center of the computer screen (panel A). Squares appeared for either short (250 ms) or long durations (750 ms), represented by the short or long line lengths (panel B). The ISI was 500 ms. For each condition, one example of each sequence type is illustrated. For the learning condition, subjects were tested on only one of the two possible sequences. Panel C contains graphs of performance measures (percent correct; coefficient of variation and response asynchrony) for the learned (LRN) and isochronous (ISO) sequences across blocks of practice (BLK1, 2 and 3). All measures showed significant improvement between BLK1 and BLK3; response asynchrony showed significant differences between all three blocks of practice. Pairwise comparisons contrasting BLK3 to ISO showed no significant differences in for any of the measures.

Stimuli and task conditions

The TMST requires subjects to reproduce a temporally complex sequence of finger taps in synchrony with a visual stimulus (Fig. 1, panel A). Visual stimuli were 10-element sequences made up of a series of white squares (3 cm^2) presented sequentially in the center of the computer screen (Fig. 1, panel B). Each sequence was preceded by a warning cue (a 2-cm^2 white square). In the learned condition (LRN), the sequence was made up of five long (750 ms) and five short (250 ms) elements with a constant inter-stimulus interval (500 ms). The sequence was constructed to have no more than two repeated elements and to have seven transitions from short to long. This results in a sequence that is temporally regular, but does not conform to a standard musical rhythm. For the isochronous (ISO) and perceptual (PER) baseline conditions sequences were made up of either all short or all long elements. The all-long and all-short sequences alternated across the block of trials. At the beginning of the testing session, subjects were given a set of practice sequences (Fig. 1, panel B) that were used to score performance for the LRN and ISO conditions. These sequences were made up of either all short or all long elements and a simple mixture (2 long, 2 short. . .). For all conditions, a trial consisted of one presentation of the particular sequence type. Each block contained 12 trials. For the LRN, ISO and PER conditions, the same number of short and long stimuli were present in each block, so that subjects received the same amount of visual stimulation and, in the LRN and ISO conditions, made the same number of motor responses. Subjects' key-press and release durations were recorded by a computer and used to calculate the three indices of learning: accuracy, response variance and response asynchrony (described in detail below). An average of 5.5 trials of each condition (trial length = 11 s) were presented during each 60-s scan.

Procedure

At the beginning of the testing session, subjects were trained on how to make the short and long key-press responses using a series of simple practice sequences (see Fig. 1, panel B, top row). For these simple practice sequences, subjects first viewed the visual stimuli for each trial type (i.e., all short; all long or the simple mixture) and were then asked to imitate three trials of each type. They were instructed to press the mouse key using the index finger of the right hand at the onset of each visual element in the sequence and to hold it for the duration of the cue, synchronizing their responses as precisely as possible to the onset and offset of the stimuli. Each subject received one block of training, for a total of 48 short and 54 long responses. Subjects were generally quite accurate, but verbal feedback was given by the experimenter after each trial on the accuracy of their responses.

After the initial training, subjects were explicitly taught the learned sequence by trial and error to a criterion of three consecutive correct repetitions. A correct repetition meant that all short and long durations of the sequence were correctly reproduced in the correct order. Verbal feedback was given by the experimenter after each trial on the accuracy of subjects' responses to guide their learning. A limit of 48 trials was given for the subject to achieve criterion. After this stage of training, subjects were not given feedback on their performance. They were, however, instructed before each block of performance in all conditions to make their responses as "precise and accurate as possible" and to "press the mouse button as each cue comes on and release it when it goes off." Subjects were then scanned while performing three blocks of the LRN condition (BLK1, BLK2 and BLK3) followed by one block of the ISO and PER conditions in counterbalanced order. Across the blocks of practice, subjects performed 36 trials of the learned sequences and 12 trials of the ISO and PER sequences.

Behavioral measures

In many motor learning tasks, such as the SRT, learning is assessed by reductions in reaction time to individual elements of the motor sequence. However, the present task required subjects to synchronize their responses as precisely as possible with the stimuli, so speeding of responses would not necessarily correspond to improved performance. Therefore, learning of the TMST was assessed by examining changes in three different variables: accuracy; variance of response durations; and synchrony of responses with target stimuli. These measures allowed us to examine learning of different aspects of the task. Accuracy reflects learning of the more explicit component of the task—encoding of the correct order of short and long durations in the sequence. However, it still requires the subject to make a relatively accurate motor response—within 2SD of his/her own baseline. Response variance reflects stabilization of the motor response, while response asynchrony reflects the subjects' ability to precisely time their key-press and key-release responses relative to the visual stimuli.

Accuracy for the LRN and ISO conditions was scored individually by using each subject's average short and long responses from the practice sequences $\pm$ 2SD as the upper and lower limits for correct response for short and long elements, respectively (Penhune and Doyon, 2002; Penhune et al., 1998). The first step in scoring was to calculate the average and SD for each subjects' long and short responses on the simple practice sequences (see Fig. 1, Panel B, top row). Responses on the simple practice sequences that were greater than 2SD from the mean were excluded. The average was then recalculated, and the recalculated average $\pm$ 2SD was used as the upper and lower limit for accurate response on the LRN and ISO sequences. For example, if a subject's average long response on the simple practice sequences was 724 $\pm$ 67, then responses between 590 and 858 would be accepted as correct for the LRN and ISO sequences. The percent of correctly reproduced elements was calculated for each trial and measures of CV and asynchrony were calculated on correct responses only. This was done so that measures of these variables would not be contaminated by gross errors. Response variance was measured using the coefficient of variation (SD/Mean) of the subject's response durations. Response asynchrony was assessed by examining the total difference between stimulus onset and offset and the onset and offset of the subject's key-press responses. All behavioral measures were averaged across blocks of trials. Differences across conditions were assessed using repeated-measures analysis of variance (ANOVA) with Greenhouse–Geiser correction. Significant differences were analyzed using tests of simple main effects with Bonferroni correction for multiple comparisons.

Scan acquisition and data analysis

PET scans were acquired using the O^{15} water-bolus method (60 s scans, Siemens HR+, 3D acquisition) resulting in a volume of 63

slices with an intrinsic resolution of 4.2 × 4.2 × 4.0 mm. T1-weighted MRI scans were acquired for all subjects (1 × 1 × 1 mm, 140–160 sagittal slices). Field of view of the PET camera allowed visualization of the entire cortex and cerebellum. MRI and PET data were coregistered (Woods et al., 1993) and automatically resampled (Collins et al., 1994) to fit the standardized stereotaxic space of Talairach and Tournoux (1988) as defined by the MNI 152 template. PET volumes were normalized, reconstructed with a 12-mm Hanning filter and averaged across subjects for each condition.

Paired image subtraction

Differences across blocks of learning were first assessed using paired-image subtraction where statistically significant peaks were identified by an automatic algorithm with a threshold set at $t \geq \pm 3.5$ (Worsley et al., 1992, 1996). Contrasts were made across BLK1-3 of learning and between BLK1 and the ISO and PER baselines. Contrasts across blocks would reveal brain regions that were differentially active across blocks of learning. Contrasts between BLK1 and the ISO baseline would reveal brain regions involved in early learning of a temporally complex sequence. The ISO baseline was selected because it requires similar timing and sensorimotor integration components as the LRN condition, but does not require learning of the more complex sequence of movements. BLK1 of learning was also contrasted with the PER baseline. This contrast would reveal all brain regions active in generating the complex movement sequence, including those potentially masked in the contrast with the ISO baseline. Finally, the ISO condition was contrasted with the PER baseline. This contrast would reveal the brain regions active during performance of the simple ISO sequences. The results of this contrast could then be compared with those active in the LRN vs. ISO contrast, to identify regions specifically active in generating the more complex response.

Normalized blood flow analyses

Results of paired image subtraction were corroborated by analyzing changes in normalized cerebral blood flow (nCBF) values from volumes of interest (VOI) for selected regions identified in the subtraction analyses. Spherical VOIs (radius 5 mm) were defined using the Talairach locations of specific significantly active regions. Average nCBF values for individual subjects were extracted for each VOI for the LRN condition for Blocks 1, 2 and 3. These values were submitted to repeated-measures ANOVA and significant differences were analyzed using tests of simple main effects with Bonferroni correction for multiple comparisons.

Behavioral regression analyses

Regression analyses were conducted to assess the relationship between behavioral variables (percent correct, CV and asynchrony) and blood flow in the three blocks of learning. For these analyses behavioral measures of percent correct, CV and asynchrony for each subject for each block of learning were regressed against nCBF for the whole brain BLK1, 2 and 3. These analyses are entirely data-driven and are independent of any baseline condition. The relationship between each behavioral measure and nCBF was assessed using analysis of covariance (ANCOVA), with subjects as the main effect and the behavioral measure as the covariate. Significance was evaluated using 3D Gaussian random field theory which corrects for multiple comparisons across the volume. Maps of the regression analyses were generated (see Fig. 4, panel A) and values equal to or exceeding 3.5 were considered significant ($P < 0.01$ two-tailed) (Worsley et al., 1992, 1996). Following this, nCBF values for the peak locations identified in the regression analyses were extracted for each subject. The graphs in Fig. 5 present the average extracted nCBF values for each ROI plotted against the average value of each behavioral measure for each block of learning.

Inter-regional correlation analyses

In order to further explore the results of the behavioral regression analyses, inter-regional correlation analyses were performed. In these analyses, the regions identified in the behavioral regression analyses were themselves used as regions of interest (ROIs) and regressed against nCBF values for the three blocks of learning. The relationship between individual-subject values of nCBF in each ROI and nCBF in the whole brain for each block of learning was assessed using analysis of covariance (ANCOVA), with subjects as the main effect and nCBF in the ROI as the covariate. As with the behavioral regressions, significance was evaluated using 3D Gaussian random field theory which corrects for multiple comparisons across the volume. Maps of the regression analyses were generated (see Fig. 4, panel B) and values equal to or exceeding 3.5 were considered significant ($P < 0.01$ two-tailed) (Worsley et al., 1998).

For all analyses, activations identified as being in the same brain region that were located within 10 mm of each other were considered to be indistinguishable, and the location of the peak with the higher t value is reported in the table. The location of active regions in the cerebellum was identified using a 3D atlas of the human cerebellum in stereotaxic space (Schmahmann et al., 2000). The location of active regions in the Dentate Nucleus (DN) was identified using an MRI atlas of the cerebellar nuclei (Dimitrova et al., 2002).

Results

Behavioral data

Subjects were able to learn the TMST sequences relatively quickly (Average 16 ± 8 trials to criterion). No subject failed to learn the sequence within the criterion training limit of 48 trials. For the LRN sequences, there was a significant improvement in performance across the three blocks of learning for all variables [see Fig. 1, panel C (percent correct: $F_{(2,22)} = 5.0$; $P = 0.02$; response variance: $F_{(2,22)} = 20.8$; $P < 0.001$; and response asynchrony: $F_{(2,22)} = 17.9$; $P < 0.001$)]. All measures showed significant differences between Blocks 1 and 3; with response asynchrony being the most sensitive, showing significant differences between all three blocks of practice. Pairwise comparisons contrasting the last block of learning to the isochronous baseline revealed no significant differences in performance for any of the measures, indicating that the baseline task was performed at the same level as the learned sequence.

Paired-image subtraction

BLK1 vs. ISO and BLK1 vs. PER

In order to identify regions that were active during early learning of the TMST, performance during the first block of practice on the learned sequences was contrasted with performance

of the ISO and PER baselines. Regions that were significantly more active during BLK1 of performance compared to the ISO baseline were found in bilateral cerebellar cortex, SMA and pre-SMA (Picard and Strick, 1996) and bilateral superior temporal gyrus. Activity in the cerebellum was seen medially in lobules V/VI and VIIIa, and laterally in lobules VI, left Crus I/II in the horizontal fissure and right VIIIa (Fig. 2, panel A and Table 1). Because the ISO baseline also involves learned sequences, BLK1 was also compared with the PER baseline which required no learning and no motor response (Fig. 2, panel B and Table 2). Results showed activations in the identical regions of the cerebellum seen in the BLK1 vs. ISO contrast, indicating that the pattern of activation observed in this contrast was not dependent on the ISO baseline. Additional regions of activity related to the motor response were seen in M1/S1, SMA/pre-SMA, bilateral superior parietal lobule, the cingulate motor area and thalamus. Cerebellar activity in lobules V, VI and VIIIa in both contrasts is consistent with the location of cerebellar connections with M1 identified in neuroanatomical studies in monkeys (Kelly and Strick, 2003), and these regions are typically seen to be active in neuroimaging studies during performance of simple finger movement tasks. The pattern of active cerebellar regions also replicates findings from a previous experiment for the same contrast of the first block of learning with the ISO baseline (Penhune and Doyon, 2002).

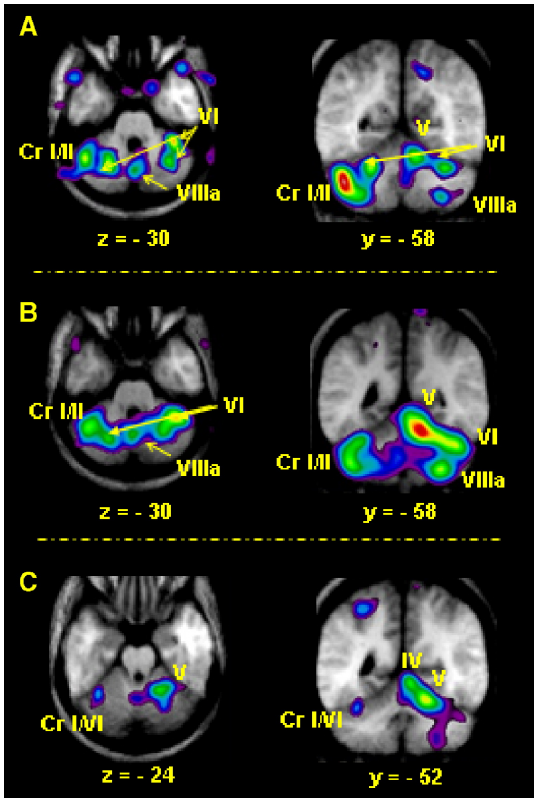

Fig. 2. Shows t statistic maps of significantly active regions in the cerebellum for the BLK1 vs. ISO contrast (panel A); the BLK1 vs. PER contrast (panel B) and the ISO vs. PER contrast (panel C). PET data are co-registered with the average MRI of the 12 subjects and slice levels are given in the standardized space of Talairach and Tournoux. Images are thresholded at $t > \pm 2.5$ to allow visualization of regions of activation below the statistical threshold of $t > \pm 3.5$ that may be of interest.

Table 1
Locations of significant differences for the BLK1 vs. ISO and the BLK3 vs. BLK2 contrasts

	x	y	z	t value
BLK1 > ISO				
R V	10	−58	−20	5.0[a]
L VI	−22	−64	−26	5.5[a]
R VI	38	−40	−32	5.7[a]
R VI	34	−60	−28	4.9[a]
L Crus I/II	−42	−52	−44	6.6[a]
R VIIIa	30	−54	−50	4.5[a]
M VIIIa	4	−70	−36	4.8[a]
SMA/pre-SMA	−2	−2	70	4.4[a]
L STG (38)	−50	16	−18	3.6
R STG (38)	44	16	40	4.0
Subcallosal gyrus (25)	2	16	−14	4.5
Precuneus	0	−72	58	3.9
R Precuneus/M Area 7	16	−66	48	3.7
ISO > BLK1				
L M1	−14	−20	74	4.6
L M1/S1	−50	−18	42	3.9
R M1/S1	50	−12	46	4.0
L Area 6/8	−32	14	50	5.2
L Area 6/8	−14	26	54	4.7
R Area 6/8	34	20	46	5.0
M Area 8	−6	38	44	4.5
L SPL (7/40)	−36	−46	50	4.8
L SPL (7)	−28	−44	66	3.5
R Area 10/46	44	40	0	5.6
L Area 10/11	−32	50	0	5.4
BLK 3 > BLK2				
L M1/S1	−16	−32	54	4.6[a]
L M1/S1	−36	−20	50	3.8[a]
L Caudal PMC	−12	−18	74	3.6[a]
R Putamen	20	12	2	3.6[a]
L IPL (7/40)	−38	−34	44	3.8[a]
R S1/SPL (7)	18	−42	70	3.8
BLK2 > BLK3				
L Crus I/II	−18	−76	−36	4.2

[a] VOIs for nCBF extractions.

ISO vs. PER

In order to better understand the contribution of the ISO condition to the BLK1 vs. ISO contrast, ISO was also contrasted with the PER baseline (Fig. 2, panel C and Table 2). This contrast showed significant regions of cerebellar activity in right lobules IV and V, and in left Crus I/VI. Additional regions of activity related to the motor response were seen in M1/S1, bilateral PMC, left inferior and superior parietal lobules and the thalamus. The pattern of cerebellar activity in this contrast replicates findings from a previous experiment for a similar contrast between an isochronous sequence condition and a perceptual baseline (Penhune et al., 1998). When compared with the previous analyses, the right lobule V and left Crus I/VI regions seen be active in the ISO vs. PER contrast showed additional activity in the BLK1 vs. ISO and BLK1 vs. PER contrasts, indicating that they were more active during learning of the more complex sequence. Further, the BLK1 vs. ISO and BLK1 vs. PER contrasts showed activity in left Crus I/II and right VIIIa that was not present in the ISO vs. PER contrast. Taken together, these results suggest that lobules IV, V and left Crus I/VI

Table 2
Locations of significant differences for the BLK1 vs. PER and the ISO vs. PER

	x	y	z	t value
BLK1 > PER				
R V	14	−54	−18	9.4
R VI	38	−46	−30	7.6
R VIIIa/b	28	−54	−50	7.2
L Crus I/II	−44	−54	−40	7.2
L Crus I/VI	−22	−66	−30	6.3
L M1/S1	−38	−28	66	6.1
SMA	−8	−8	66	5.9
SMA/PMC	10	0	72	5.7
SMA	−2	0	54	5.1
L Thalamus (DM)	−2	−16	6	4.5
L SPL (7)	−18	−62	62	4.0
R SPL (7)	18	−68	44	3.9
Rostral cingulate motor (32)	−6	16	42	3.6
PER > BLK1				
L Frontal (47/11)	−40	42	−10	7.8
L Frontal (8)	−14	24	56	6.7
L Frontal (6/8)	−32	16	46	6.6
L Frontal (8)	−24	32	42	5.4
L Frontal (45/46)	−46	30	12	5.3
L MTS (22/21)	−60	−44	−4	4.9
L Parietal (7/40)	−50	−54	42	4.7
R Frontal (47/11)	38	38	−4	4.6
R Frontal (8)	18	40	46	4.4
L Frontal (10)	−10	56	26	4.1
R M1/S1	48	−12	46	3.8
R Frontal (8)	34	20	44	3.8
R HG	52	−10	0	3.4
ISO > PER				
R V	18	−52	−18	6.2
R IV	6	−52	−6	5.6
L VI/Crus I	−36	−54	−26	4.1
L M1/S1	−34	−24	58	7.7
L Caudal PMC	−12	−14	64	6.9
L M1/S1	−32	−24	68	6.5
L S1/IPL (40)	−50	−26	52	5.7
R Rostral PMC	14	−2	56	5.4
L Intra-parietal sulcus (7/40)	−32	−54	−26	5.2
L Thalamus (DM)	−2	−24	6	4.5
R Rostral PMC	12	0	74	4.0
L SPL (7)	−16	−58	62	3.5
PER > ISO				
L Parietal (7/39)	−48	−66	30	4.2
L Frontal (47/11)	−42	38	−8	4.0
L Frontal (44/45)	−52	20	6	4.0
R Med Orb Fron (47)	8	12	−18	3.8
L Frontal (8)	−26	22	42	3.5
L Inf Temporal	−44	−16	−26	3.5
Subcallosal cingulate	−4	34	−14	3.5
R Cuneus (7)	12	−48	48	3.5
R Frontal (8)	22	38	46	3.5

are involved in performance of a simple timed motor sequence. Because a similar number of movements were made in the LRN and ISO conditions, the additional activity in left Crus I/II and right VI and VIIIa seen in the BLK1 contrasts is likely to be related to early learning and optimization of specific kinematic parameters of the more complex sequence, rather than basic sequencing or motor output per se. SMA/pre-SMA was also seen to be active only in the BLK1 contrasts, consistent with a role in the production of a more complex sequence of movements.

BLK3 vs. BLK2

Comparison of BLK3 to BLK2 showed no additional activity in the cerebellum (Fig. 3, panel A and Table 1). Regions of greater activity in BLK3 were seen in contralateral M1 and PMC, the right putamen, superior and inferior parietal lobules and orbital frontal cortex [areas 47/12 of Petrides; (Chiavaras and Petrides, 2000)]. These results are consistent with the hypothesis that as a motor sequence is better learned, the cerebellum is less actively involved

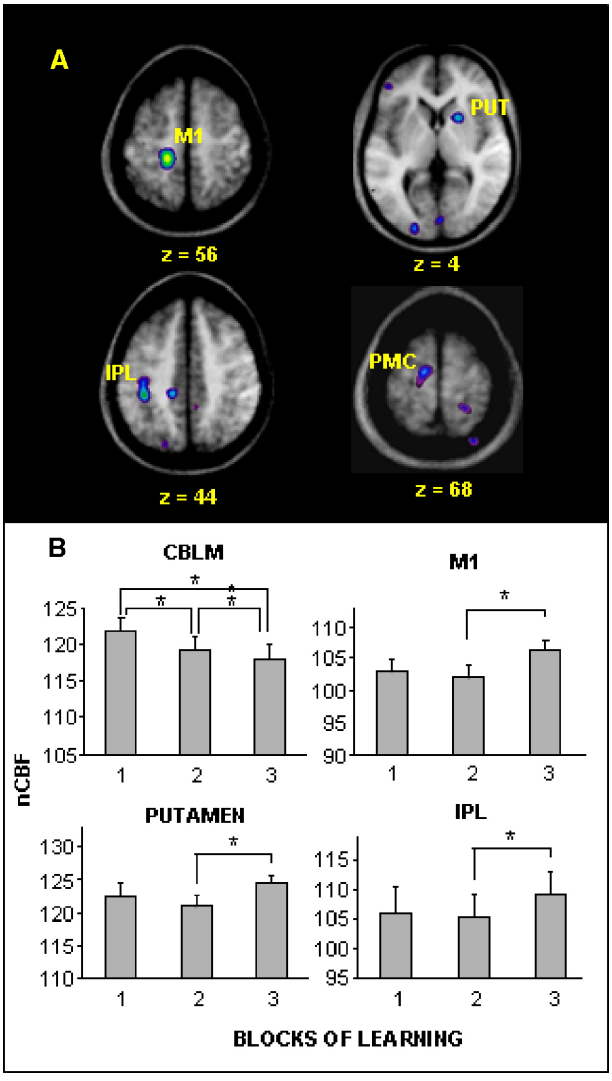

Fig. 3. Panel A shows *t* statistic maps of significantly active regions in M1, the Putamen, PMC and IPL (Area 40) for the BLK3 vs. BLK2 contrast. PET data are co-registered with the average MRI of the 12 subjects and slice levels are given in the standardized space of Talairach and Tournoux. Images are thresholded at $t > \pm 2.5$ to allow visualization of regions of activation below the statistical threshold of $t > \pm 3.5$ that may be of interest. Panel B shows graphs of nCBF values extracted from VOIs in the cerebellum, M1, Putamen and IPL for BLK1-3 (For VOI locations, see Table 1). All regions showed significant differences between BLK2 and BLK3. The cerebellum showed significant differences between all three blocks of learning.

in production of the response and that the BG and other motor-related cortical areas become more important (Doyon et al., 2003).

Normalized blood flow analyses

In order to directly examine blood flow changes across blocks of learning, nCBF values were analyzed for specific VOIs based on active regions identified in the subtraction analyses (Fig. 3, panel B). These analyses are based on nCBF values for the BLK1, BLK2 and BLK3 scans only and are not dependent on the ISO baseline. VOIs for the selected regions were centered on the Talairach location of the highest t value for each region (for locations, see Table 1). Average nCBF values for each VOI were extracted from the BLK1, BLK2 and BLK3 scans. These values were submitted to repeated measures ANOVA to examine changes in nCBF values across blocks of learning and to compare the learning and isochronous baseline conditions.

In the cerebellum, VOIs were created for the seven regions that were active in the BLK1-ISO1 contrast. Across BLK1, 2 and 3, there was a significant main effect of Block, such that nCBF decreased across blocks of learning ($F_{(2,22)} = 20.4$; $P < 0.001$). Tests of simple main effect showed that averaged across the seven regions, nCBF in the cerebellum decreased significantly across all three blocks ($P < 0.05$). Examination of individual cerebellar regions showed that the majority showed significant decreases between BLK1 and BLK3, with only left Crus I/II and right VI showing consistent decreases across all three blocks. Only right medial lobule V showed no significant change across blocks. This suggests that left Crus I/II and right VI may be most crucially involved in learning and that right lobule V may play a role in production of the motor response, regardless of the level of learning.

Changes in M1, the putamen, PMC and IPL were examined for VOIs based on the peaks of activation observed in the BLK3 vs. BLK2 contrast; changes in SMA/pre-SMA were examined for a VOI based on the peak of activation observed in the BLK1 vs. ISO subtraction (Fig. 3, panel B; for locations of VOIs, see Table 1). Results of separate ANOVAs showed significant main effects of Block for M1 ($F_{(2,22)} = 15.7$; $P < 0.0001$) and IPL ($F_{(2,22)} = 4.3$; $P < 0.03$), and a marginally significant effect for the Putamen ($F_{(2,22)} = 3.5$; $P < 0.06$). No significant effects were observed for the SMA ($F_{(2,22)} = 2.4$; $P > 0.05$) or PMC ($F_{(2,22)} = 1.0$; $P > 0.05$). Tests of simple main effect showed significant increases in nCBF from Block 2 to Block 3 for M1 ($P = 0.003$), the Putamen ($P = 0.02$) and the IPL ($P = 0.02$). Taken together, these results show that the M1, the IPL and Putamen are more active during the last block of learning, when performance has improved. Because the stimuli and sequences to be performed are identical for each block, these results demonstrate that activity in these regions is specifically related to learning of the task rather than any differences in task parameters.

Behavioral regression analyses

In order to directly examine the relationship between task performance and brain activity, these analyses regressed individual behavioral measures for BLK1, 2 and 3 against nCBF across the entire brain for each block. Regression analyses are particularly powerful because they are entirely data-driven. Further, they identify changes in brain activity in relation to performance changes in identical scan conditions and do not depend on any baseline condition. Results showed that better task performance was correlated with greater CBF in M1 and the pre-SMA, while poorer performance was correlated with greater CBF in left Crus I/II, right VI/Crus I and the right DN of the cerebellum (see Fig. 4, Panel A and Table 3). Greater cerebellar activation while performance is poor is consistent with models suggesting that the cerebellum is involved in detection of movement errors at the beginning of the learning process. The regions of significant correlation were consistent for all three performance measures. The left Crus I/II and right VI/Crus I locations correlated with poorer performance were within 10 mm of those showing greater activity in the BLK1 vs. ISO comparison. The M1 locations correlated with better performance were also very similar to those observed in the BLK3 vs. BLK2 comparison. nCBF values for the left Crus I/II and M1 locations identified in the regression analyses were extracted for each subject and the average values for each ROI are plotted against the average value of each behavioral measure for each block of learning showing the relationship between behavioral and blood flow changes (see Fig. 5).

Inter-regional regression analyses

Although the behavioral regression analyses suggest an interaction between the cerebellum and M1 during learning, they do not directly assess how changes in blood flow in the two regions are related to each other, or to other regions of the brain. In order to examine this question, regions of interest (ROIs) were identified based on the results of the behavioral regression in left Crus I/II, right lobule VI/Crus I and left M1 (see Table 3 for locations). nCBF in these regions for BLK1, 2 and 3 were then regressed against nCBF in the whole of the brain for the same scans. Inter-regional regression analyses identify any brain region whose blood flow is related to blood flow in the ROI and do not depend on a priori knowledge of possible relationships between brain regions. The results of these analyses extended the results of the behavioral analysis by showing a negative correlation between blood flow in left Crus I/II and right lobule VI/Crus I of the cerebellum and blood flow in M1 (see Fig. 4, Panel B and Table 4). Activity in right lobule VI/Crus I was positively correlated with activity in right DN, the primary output pathway to M1. Anatomical studies in monkeys have shown that lobule VI projects through the dorsal DN to M1 (Kelly and Strick, 2003). Further, activity in left Crus I and right lobule VI/Crus I was positively correlated with activity in right DN, and each region was positively correlated with the other. Activity in M1 was positively correlated with superior and inferior parietal regions, and negatively correlated with activity in left Crus I/II, right VI/Crus I and the right DN. When combined with the results of the behavioral regressions, these analyses show that early in learning, when performance is poor, activity in the cerebellar hemispheres and DN is high. As performance improves, cerebellar activity decreases, while blood flow increases in M1 and parietal regions. This pattern of results suggests that the interaction between the cerebellum and motor cortical regions may play a role in the laying down of motor memories that are stored in M1.

Discussion

The results of this experiment demonstrate an interaction between the cerebellum and motor cortex during within-day

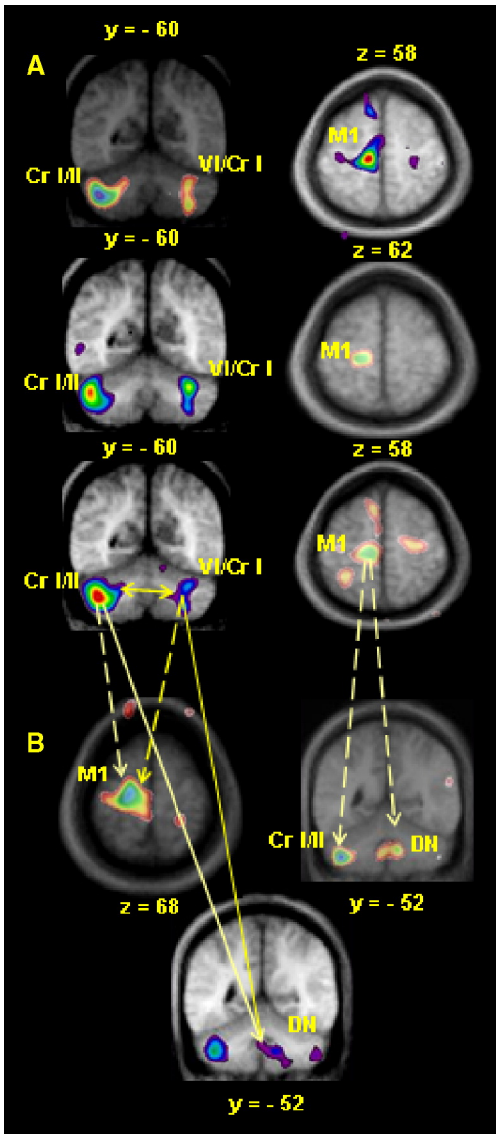

Fig. 4. Panel A presents *t* statistic maps of regions in the cerebellum and M1 that showed significant correlations with behavioral variables across BLK1-3 of learning. Top row = regions correlated with percent correct; middle row = regions correlated with the coefficient of variation; bottom row = regions correlated with response asynchrony. Panel B illustrates the inter-regional correlation analyses, showing the regions whose activity correlated with the ROIs in left Crus I/II, right VI/Crus I and M1 (For ROI locations, see Table 3). Solid lines represent positive correlations and broken lines represent negative correlations. PET data are co-registered with the average MRI of the 12 subjects and slice levels are given in the standardized space of Talairach and Tournoux. Images are thresholded at $t > \pm 2.5$ to allow visualization of regions of activation below the statistical threshold of $t > \pm 3.5$ that may be of interest.

learning of a motor sequence task. Contrasts across blocks of practice show that the cerebellum was most active during early learning, while M1, IPL and the putamen were more active during later learning. These results were corroborated by analyses showing that nCBF in the cerebellum decreased significantly across blocks of practice, while blood flow in M1, IPL and the putamen increased between Blocks 2 and 3. This pattern of greater cerebellar activity early in learning with a switch to greater activity in the M1 and the BG later in learning is consistent with models of

Table 3
Locations of significant regions for the behavioral regression analyses

Location	*x*	*y*	*z*	*t* value
Positive correlations with percent correct				
L M1	−10	−22	58	5.4[a]
L M1/S1	−56	−14	40	4.0
L M1/S1	−42	−26	46	3.8
L M1	−52	−8	22	3.7
L SPL (2/40)	−28	−30	42	3.7
R SPL (7/40)	30	−38	50	3.5
pre-SMA	−10	22	56	3.6
SMA	−4	−8	54	3.3
L HG (42)	−48	−24	8	3.3
Negative correlations with percent correct				
L Crus I/VI	−22	−68	−32	5.5[a]
L Crus I/II	−40	−62	−42	5.2
R Crus I/II	34	−66	−40	4.0[a]
R Dentate	10	−52	−40	3.8
R Thalamus (DM)	4	−18	12	4.5
R IPL (40)	62	−38	40	3.8
Anterior cingulate (32)	2	48	12	3.8
R Inferior Temporal Sulcus	54	−22	−16	3.7
Positive correlations with				
L Crus I/II	−44	−56	−34	5.3[a]
L Crus I/VI	−36	−70	−24	3.8
R VI/Crus I	34	−60	−32	4.4[a]
R PMC	10	6	72	4.5
Cingulate arm area	0	24	30	4.1
R Uncus	24	4	−26	3.6
Negative correlations with CV				
L M1	−16	−26	62	4.2[a]
M Area 9	−8	48	32	3.7
Medial orbital sulcus	6	58	−16	3.5
L HG (42)	−46	−20	8	2.7
Positive correlations with asynchrony				
L Crus I/II	−40	−62	−40	6.1[a]
L Crus I	−40	−70	−26	4.9
R Dentate	12	−52	−42	4.4
R VI/Crus I	36	−66	−30	4.1[a]
R Crus II	30	−62	−48	3.5
Thalamus (DM)	6	−20	14	4.0
Anterior Cingulate (25)	6	30	2	3.9
Cingulate motor area	−6	20	40	3.7
Gyrus rectus (25)	0	18	−16	4.0
R anterior insula	30	14	−12	3.7
R Med Orbital Frontal Gyrus	24	14	−20	3.5
Negative correlations with asynchrony				
L M1	−12	−24	58	5.2[a]
L M1/S1	−32	−30	44	5.1
L M1/S1	−20	−26	44	4.3
L S1	−54	−20	40	4.2
R M1	26	−18	60	3.9
pre-SMA	−6	18	58	4.0
L SPL (5/7)	−28	−46	62	4.4
L HG (42)	−48	−20	10	3.5

[a] Location used as VOIs for nCBF extractions plotted in Fig. 5 and to create ROIs for inter-regional correlation analyses.

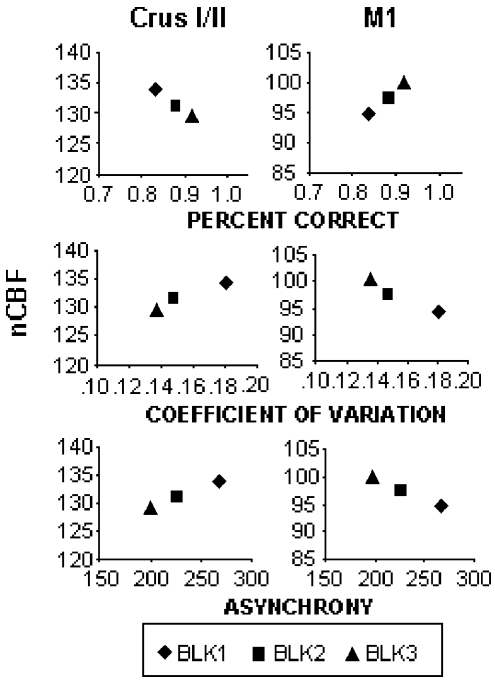

Fig. 5. These graphs plot average extracted nCBF values in Crus I/II and M1 for BLK1-3 against the average for each behavioral variable for BLK1-3. Top panel = percent correct; middle panel = coefficient of variation; and bottom panel = asynchrony. nCBF values for each subject were extracted from VOIs in left Crus I/II and M1 centered around the peaks identified in the regression analyses (For VOI locations see Table 3).

motor sequence learning (Doyon et al., 2003) and is strikingly similar to that observed in a previous study of across-day learning using the same task (Penhune and Doyon, 2002). This suggests that the network of regions that contribute to early learning may be similar to those that contribute to long-term learning and memory. Behavioral regression analyses showed that poorer task performance was correlated with activity in left Crus I/II, right lobule VI/Crus I and the DN of the cerebellum, while better performance was correlated with activity in M1. Inter-regional correlation analyses confirmed that as blood flow in the cerebellum decreases, blood flow in M1 increases. Further, activity in the dentate was positively correlated with activity in the cerebellar hemispheres and negatively correlated with activity in M1. This indicates that early in learning, when performance is poor, activity is greatest in the cerebellar hemispheres and the dentate. Activity in the hemispheres is likely related to mechanisms which optimize movement kinematics resulting in behavioral improvement. Activity in the dentate may be related to its role in consolidation processes that lay down motor memories (Doyon et al., 2002; Nezafat et al., 2001). As task performance improves, blood flow in the cerebellar hemispheres and dentate decreases, while activity increases in M1. Greater M1 activity may reflect the strengthening of synaptic connections associated with changes in motor maps (Classen et al., 1998; Pascual-Leone et al., 1994).

Cerebellum–M1 interactions during early learning

These results are the first to show a direct relationship between decreasing activity in the cerebellar cortex and increasing activity in motor cortex with learning of a motor sequence task. Previous studies have suggested such a relationship by showing decreases in cerebellar activity along with increases in M1, SMA or other motor cortical regions in the same experiment (Doyon et al., 2002; Nezafat et al., 2001; Penhune and Doyon, 2002; Toni et al., 1998;

Table 4
Significant locations for the inter-regional correlation analyses

Location	*x*	*y*	*z*	*t* value
Positive correlations with left Crus I/II				
R VI/Crus I	36	−68	−28	4.6
R VI	36	−38	−32	3.9
M VIIb	2	−70	−32	3.7
R Dentate	14	−52	−42	3.6
R Rostral PMC	16	14	60	3.9
R Thalamus (DM)	4	−20	10	4.6
R GP/Putamen	26	−8	−4	3.7
M Area 9	4	44	26	3.9
Subcallosal cingulate	4	18	−12	3.7
R Occipito-parietal (19/39)	44	−72	28	4.3
R Inf Temporal Gyrus (21)	62	−8	−28	4.1
R Inf Temporal Sulcus	52	−22	−16	4.1
R Anterior Insula	40	20	−6	3.7
Negative correlations with left Crus I/II				
L M1	−14	−20	68	5.4
M M1	−6	−34	60	4.9
L M1/S1	−32	−26	60	4.6
L M1/S1	−56	−14	42	4.2
L M1/Area 43	−56	−8	12	3.7
R M1/S1	28	−26	60	3.6
Pre-SMA	−2	10	56	4.1
Posterior cingulate	14	−30	40	3.7
Posterior cingulate	−8	−38	30	3.7
L Parahippocampal gyrus	−32	−52	−6	3.9
Area 17	0	−66	6	3.9
Area 19	−28	−92	20	3.6
Positive correlations with right VI/Crus I				
L Crus I/II	−38	−66	−38	5.2
L Crus I/II	−42	−54	−46	4.7
R Dentate	10	−50	−40	4.3
L Crus I/II	−26	−48	−46	4.2
Negative correlations with right VI/Crus I				
L M1	−10	−24	58	4.7
L M1(inferior)	−54	−12	32	4.0
Positive correlations with left M1				
R M1	40	−12	52	3.5
L SPL (5/7)	−30	−42	66	4.8
L IPL (2/40)	−50	−30	36	4.1
R SPS (7/40)	36	−40	46	3.7
L SPL (7)	−20	−76	46	3.5
L Area 17	2	−76	16	3.7
Negative correlations with left M1				
L Crus I/II	−22	−72	−34	5.7
L Crus I/II	−40	−60	−42	5.7
R Crus I	34	−64	−36	4.0
L Crus I/VI	−38	−68	−24	3.6
M IX	−4	−50	−42	3.5
R Dentate	10	−52	−40	3.8
R Thalamus (DM)	4	−20	10	4.2
Subcallosal cingulate	−2	24	−14	4.2
R Anterior insula	28	12	−14	4.8
M Frontal Gyrus (9/10)	4	48	18	4.6

Van Mier et al., 1998). This interaction is consistent with the model of Doyon et al. (2003), proposing that the cortico-cerebellar system is crucial for early learning of motor sequence tasks. Decreasing activity in cerebellar cortex with practice or learning has been observed in a large number of previous neuroimaging studies (Doyon et al., 2002; Flament et al., 1996; Nezafat et al., 2001; Toni et al., 1998; Van Mier et al., 1997, 1998), as has the finding of increased activity in M1 with greater practice (Classen et al., 1998; Karni et al., 1995; Nudo et al., 1996; Pascual-Leone et al., 1994; Penhune and Doyon, 2002). Activity in the hemispheres is likely related to mechanisms which optimize movement kinematics resulting in behavioral improvement. Decreasing activity in cerebellar cortex with learning has been hypothesized to be related to reduction in climbing fiber input resulting from decreasing error signal as learning proceeds. This pattern of decreasing activity has also been observed in electrophysiological studies in animals during learning of forelimb tasks (Bloedel et al., 1993; Gilbert and Thach, 1977; Ojakangas and Ebner, 1992). Increased activity in M1 with learning has been hypothesized to be related to changes in connectivity and synaptic strength related to practice and later storage of motor patterns (Kleim et al., 2004; Nudo et al., 1996).

The results of the inter-regional regression analyses provide further evidence for an interaction between the cerebellum and motor cortex during learning that is mediated by the DN. These analyses showed a positive correlation between activity in left and right cerebellar hemispheres and a negative correlation with activity in M1. They also showed that activity in the DN was positively correlated with activity in the hemispheres and negatively correlated with activity in M1. Based on known cerebellar physiology and connectivity (Medina et al., 2002; Ohyama et al., 2003), increased activity in the cerebellar cortex during early learning is thought to be related to greater climbing fiber input related to greater error signal. This would lead to greater activity at the level of the Purkinje cells, producing greater inhibition, and reduced CBF in the DN. As learning proceeds and movement becomes more automatic, error signal and thus Purkinje cell activity should diminish, leading to a release of inhibition, and increased CBF in the DN. All of this should produce a negative relationship between cerebellar cortical and DN activity during later learning, which has been observed in a number of previous human and animal studies (Doyon et al., 2002; Mauk et al., 2000; Nezafat et al., 2001). In a recent fMRI study of eyeblink conditioning in rabbits, Miller et al. (2003) showed a negative correlation between cortex and DN activity, but also showed that as the level of DN activity increased, the area of response shrank. They interpreted this as indicating that the region of response in the DN became more selective with learning.

In the present experiment, we saw a positive correlation between activity in the cerebellar hemispheres and the DN, indicating that DN activity decreased across blocks of learning. Based on the evidence described above, there are two possible explanations for this pattern of activity. First, it is possible that activity in the cortex and DN may be more similar during the very early phase of learning studied in our experiment, than in the later phases of learning examined in other experiments (Doyon et al., 2003; Miller et al., 2003). Second, given the relatively low resolution of PET, it is possible that the apparent decrease in DN activity is the result of a shrinking area of activity, as observed in the Miller et al. study. Future fMRI studies of early learning, with a single-trial design and higher resolution, should allow us to disentangle these two alternatives.

Similar networks for early and long-term learning

Interestingly, the observed pattern of cerebellar and M1 interaction within a single day of learning is similar to that found in a previous study of long-term learning of the same task (Penhune and Doyon, 2002). In that experiment, cerebellar activity was reduced after 5 days of learning, while M1 activity was greater at 4-week delayed recall. Taken together with the present results, this suggests that the network of regions that contribute to early learning may be similar to that which contributes to long-term learning and retention. As described in Introduction, the cerebellum and M1 have been implicated in both short-term and long-term learning of motor tasks. Based on experiments with rats, Kleim et al. (2002b, 2004) has proposed that short-term learning, in both M1 and the cerebellum, is supported by strengthening of connections, while long-term learning is mediated by synaptogenesis. Importantly, the present results indicate that these processes appear to involve similar interactions between the cerebellum and motor cortex in an on-going process of learning and consolidation. Shadmehr et al. have proposed that increasing activity in the dentate may be related to consolidation and long-term encoding of motor memory, and that as learning proceeds, the typical changes in M1 organization and response patterns may be related to changed input from the DN (Nezafat et al., 2001).

Cerebellar mechanisms mediating early learning

These results are also important in showing that changes in activity in the cerebellum and M1 are directly related to behavioral measures of learning. Regression analyses showed that better performance in terms of response stability (CV) and response synchronization was positively correlated with activity in M1 and negatively correlated with activity in the cerebellum. Both Nezafat et al. (2001) and Flament et al. (1996) showed that improved performance was negatively correlated with activity in the cerebellar cortex. However, a direct relationship between performance and plasticity in M1 has only been observed in animals (Kleim et al., 2002a, 2004), with human studies providing only indirect evidence (Classen et al., 1998; Muellerbacher et al., 2002; Pascual-Leone et al., 1994). Our results suggest that cerebellar mechanisms which contribute to early learning are those involved in optimizing movement to produce an accurately timed, stable response that is calibrated to external stimuli. A large number of studies in both animals and humans have implicated the cerebellum in movement timing (Ivry, 1996; Mauk and Buonomano, 2004; Shin and Ivry, 2003) and work in experimental animals indicates that the cerebellum is important in controlling kinematic variables such as force, velocity or muscle stiffness (Greger et al., 2004; Smith, 1996; Thach, 1996) that would be important for optimizing movement in the context of motor learning. While this experiment did not directly measure such kinematic variables, it is very likely that the changes observed in accuracy, CV and response synchronization represent indirect measures of these parameters. Future experiments directly assessing the relationship between cerebellar activity and changes in kinematic variables during learning will allow the identification of specific parameters of learning that are under cerebellar control. Although the finding that behavioral measures of learning correlate with activity in the cerebellum and M1 is strong evidence that these regions are involved in learning, it is still possible that these changes in activity are affected by non-

task-specific neural processes that change over time (Rajah et al., 1998). While this is a limitation of the present design, we did not observe systematic decreases in sensory areas or the anterior cingulate, regions that have shown task-independent changes due to sensory habituation or arousal.

The correlation of behavioral measures with activity in Crus I and lobule VI strongly supports the role of the cerebellum in motor learning. In contrast, Seidler et al. (2002) have claimed that the cerebellum is not involved in learning, but only in motor performance. They examined within-day learning of the SRT under dual-task conditions and showed that cerebellar activity was seen only when the secondary task was removed and performance improved. They concluded that covert learning had occurred under dual-task conditions, and that because no cerebellar activity was observed, the cerebellum is not required for learning. However, it is also possible that use of the secondary task prevented learning itself, not just the expression of learning. In their original study with the SRT task, Nissen and Bullemer (1987) showed that subjects performed better under single- than dual-task conditions, even on the first block of training. This suggests that the behavioral change observed when the secondary task was removed may simply represent the beginning of the learning process. If this is the case, then the concurrent increase in cerebellar activity could be seen to be related to early learning of the task. Importantly, however, Seidler et al.'s results suggest that the cerebellum is not required for the learning of the movement sequence at a global level, but rather it is involved in learning specific motor parameters that result in optimization of performance.

We would argue that motor learning in the early phase can be conceptualized as nothing more than ongoing movement optimization. Therefore, what the cerebellum learns is not simply the sequences of movements to be performed, but patterns of movement kinematics which optimize performance. This is consistent with our finding of a robust relationship between cerebellar activity and behavioral variables that show improvements in the precision and accuracy of performance. This interpretation is also consistent with the work of Bloedel et al., who have shown that inactivation of the cerebellar nuclei does not prevent learning or performance of a complex motor task, but does impair on-line adaptation of movement (Shimansky et al., 2004; Wang et al., 1998). It is also consistent with the work of Thach (1996), who has proposed that the cerebellum is most important for combining individual movements and motor context into movement "synergies". On-line optimization of movement would depend on other proposed cerebellar mechanisms such as, feed-forward and error correction (Nezafat et al., 2001; Ohyama et al., 2003); development of internal models (Imamizu et al., 2003), sensorimotor integration (Bloedel, 1992; Bower, 1995) and movement timing (Ivry, 1996). Further, the present results also show that as learning continues, these cerebellar mechanisms are less necessary for producing an accurate response. Finally, they suggest that the optimized movement parameters for the learned sequence are encoded in M1 and other motor-related structures, and that the encoding of this information may be mediated by the DN.

Acknowledgments

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