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Dynamic Cortical and Subcortical Networks in Learning and Delayed Recall of Timed Motor Sequences

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We used positron emission tomography to examine learning and retention of timed motor sequences. Subjects were scanned during learning (LRN) and baseline (ISO) on 3 d: day 1, after 5 d of practice (day 5) and after a 4 week delay (recall). Blood flow was compared across days of learning and between the LRN and ISO conditions. Overall, significant changes in activity were seen across days for the LRN condition, but not the ISO baseline. Day 1 results revealed extensive activation in the cerebellar cortex, particularly lobules III/IV and VI. Day 5 results showed increased activity in the basal ganglia (BG) and frontal lobe, with no significant cerebellar activity. At recall, significantly greater activity was seen in M1, premotor, and parietal cortex. Blood flow in the cerebellum decreased significantly between day 1 and recall. These results reveal a dynamic network of motor structures that are differentially active during different phases of learning and delayed recall. For the first time our findings show that recall of motor sequences in humans is mediated by a predominantly cortical network. Based on these results, we suggest that during early learning cerebellar mechanisms are involved in adjusting movement kinematics according to sensory input to produce accurate motor output. Thereafter, the cerebellar mechanisms required for early learning are no longer called into play. During late learning, the BG may be involved in automatization. At delayed recall, movement parameters appear to be encoded in a distributed representation mediated by M1, premotor, and parietal cortex.

Key words: motor-skill learning; motor cortex; basal ganglia; PMC; cerebellum; frontal lobe; memory; human; procedural learning

Humans learn a wide variety of complex motor skills and retain them over long periods of time. Although robust long-term retention is a hallmark of motor learning, very few studies have looked at the neural structures involved in maintaining long-term representations of motor skills. Therefore, the present experiment used positron emission tomography (PET) to compare brain regions active during recall of a timed motor sequence with those active on two earlier days of learning.

A large body of literature exists related to motor-skill learning. Studies in animals and humans have shown that motor cortical regions, the cerebellum, and the basal ganglia (BG) are critically involved in learning skilled movements (Graybiel, 1995; Thach, 1996; Doyon, 1997; Karni et al., 1998; Van Mier, 2000). Current models suggest that different networks of cortical and subcortical regions are preferentially involved at the early and late phases of skill acquisition (Karni et al., 1998; Hikosaka et al., 1999; Van Mier, 2000; Doyon and Ungerleider, 2002). Neuroimaging studies of motor sequence learning have shown decreasing cerebellar activation as a task is learned, accompanied by increasing activation in the BG, primary motor cortex (M1), and the supplementary motor area (SMA) (Grafton et al., 1994; Jenkins et al., 1994; Karni et al., 1995; Doyon et al., 1996, 1999; Van Mier et al., 1997; Toni et al., 1998). Based on current evidence, Doyon and Ungerleider (2002) have hypothesized that early learning of motor sequences recruits a predominantly cerebello-cortical network, but that late learning and delayed recall may rely on a predominantly striato-cortical network. In contrast, available data on long-term retention of motor skills is sparse. Although not examining recall directly, studies of long-term practice have shown plasticity in M1 of both humans (Pascual-Leone et al., 1995; Karni et al., 1998) and monkeys (Nudo et al., 1996). Neuroimaging studies of overlearned skills, such as typing and writing, have also shown involvement of M1, along with the SMA and premotor cortex (PMC) (Seitz et al., 1994; Gordon et al., 1998). The majority of evidence shows reduced cerebellar activity in well learned tasks. However, a single study in monkeys suggests that the cerebellar nuclei may be important in delayed recall (Hikosaka et al., 1999).

In summary, both cortical and subcortical regions play important roles in motor-skill learning. However, the pattern of activity across these regions for both learning and delayed recall has not previously been examined. Therefore, in the present experiment, subjects were tested during early learning (day 1); late learning (day 5: after 5 d of practice); and delayed recall (after a 4 week delay with no additional practice). We predicted decreased cerebellar activity between days 1 and 5, with increased activation in the BG and motor cortical regions. At delayed recall, we predicted no residual cerebellar activation, and a complete shift of activity to the BG and motor cortical regions such as M1, PMC, and the SMA.

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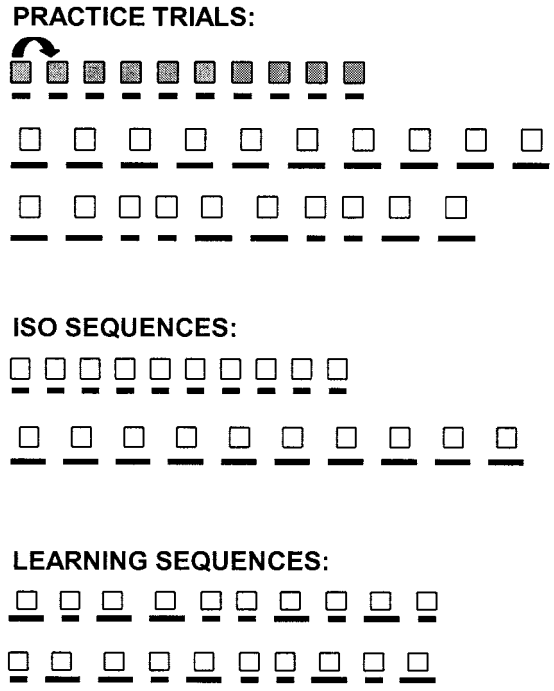

Figure 1. Illustrates the stimuli sequences used for practice and for the isochronous and learning conditions (see stimuli and task conditions). Stimulus sequences were made up of white squares that appeared sequentially at the center of the computer screen. Squares appeared for either short or long durations, represented by the short or long line lengths in the figure. 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. Blocks of practice sequences contained three repetitions of each sequence type. Blocks of isochronous sequences contained six repetitions of each sequence type. Blocks of learning sequences contained 12 repetitions of each sequence.

MATERIALS AND METHODS

Subjects. Subjects were nine healthy, right-handed volunteers selected to have not >3 years of musical training or experience (five female, four male; average age, 23.5). 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.

Stimuli and task conditions. The task used in this experiment required subjects to reproduce a complex timed motor sequence by tapping in synchrony with a visual stimulus using a single key of the computer mouse (Fig. 1). Stimuli were 10-element visual sequences made up of a series of white squares (3 cm^2) presented sequentially in the center of the computer screen. In the learned condition (LRN), two sequences and two tempos were used. Each sequence was made up of five long (750/600 msec) and five short (250/300 msec) elements with a constant interstimulus interval (500/300 msec). The two tempos and the two sequences were crossed and counterbalanced across subjects. In the LRN condition, each subject performed a single sequence at one of the two tempos. Sequences were constructed to have five short and five long elements, to have no more than two repeated elements, and to have seven transitions from short to long. This resulted in sequences that were temporally regular, but did not conform to a standard musical rhythm. In the isochronous baseline condition (ISO), 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. Each block of trials contained 12 presentations of the learned or isochronous sequences. Therefore, the same number of short and long stimuli were present in each block of the LRN and ISO conditions, so that subjects received the same amount of visual stimulation and made the same number of motor responses. The ISO condition was selected as the baseline because it requires similar timing and sensorimotor integration components as the LRN condition, but does not require learning of a complex temporal sequence. Before performing the LRN or ISO sequences on each day, subjects were given a set of practice sequences (Fig. 1) that were used to score performance on the LRN and ISO conditions. 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). We performed 5.5 trials of the LRN or ISO condition (trial length, 11 sec) during the period of each 60 sec scan.

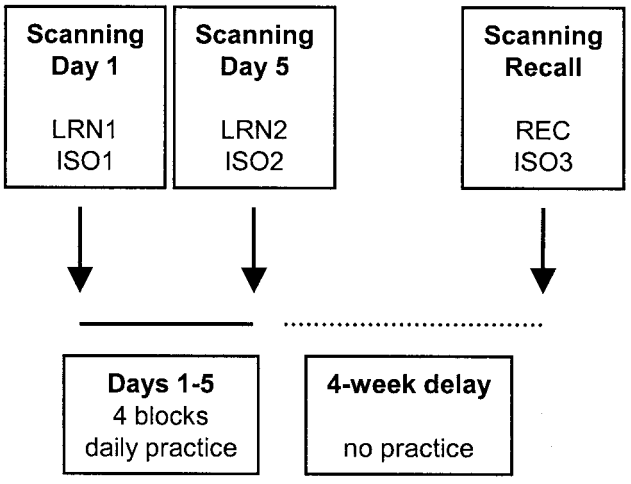

Figure 2. Illustrates the experimental design (see Materials and Methods). The *top three blocks* describe the 3 d of scanning. The *bottom two blocks* describe the days between scans.

Procedure. Each subject was scanned on three separate days (Fig. 2): day 1 of learning, after 5 d of practice (day 5) and after a 4 week delay with no further practice (recall). Two scans related to this experiment were performed on each day. On day 1, subjects were placed in the scanner and trained on the task using a set of practice sequences. They were then explicitly taught the learned sequence to a criterion of three consecutive correct repetitions. After this initial training, subjects were not given feedback on their performance. Subjects were then scanned while performing one block of the LRN condition (LRN1). Three additional blocks of practice were performed without scanning, for a total of four blocks of practice. Subjects were then scanned while performing one block of the ISO condition (ISO1). On days 2–4, subjects returned to the laboratory to perform four blocks of practice on the LRN condition without scanning. On these days, subjects performed the practice sequences, but not the ISO sequences. On day 5, subjects were placed in the scanner and performed the practice sequences and three blocks of the learned sequence without scanning. They were then scanned on the final block of learning (LRN2) and the isochronous baseline (ISO2). Across the 5 d of practice subjects performed 20 blocks (240 trials) of the learned sequences and three blocks (36 trials) of the ISO sequences. After a 4 week delay with no additional practice, subjects again returned to the lab and were scanned while performing a single block of the learned sequence (REC) and the isochronous baseline (ISO3). Subjects were specifically instructed not to practice the learned sequence during the 4 week delay and were debriefed on the final day of scanning to be sure that they had complied with that instruction. No subject reported practicing during the delay.

Behavioral measures. In typical motor sequence tasks, learning is assessed by changes in error, speed, or reaction time. In an explicitly learned sequence, errors usually decrease quickly, so reaction time is the parameter most frequently used to measure learning. However, because timing was the parameter of interest in this experiment, learning could not be assessed by decreases in reaction time. Therefore, learning of the present task was assessed by examining changes in three different variables: accuracy, variance of response durations, and synchrony of responses with target stimuli. Accuracy was expected to improve quickly, whereas the other variables were expected to change more slowly over the course of learning. Accuracy for the LRN and ISO conditions was scored individually by using each subject's average short and long responses from the practice sequences for each day ± 2 SD as the upper and lower limits for correct response for short and long elements, respectively. Percentage of correct values were calculated for each trial (for additional details on the scoring method see, Penhune et al., 1998). Response variance measured the stability of the subject's response, by calculating the coefficient of variation (SD/mean) of the subject's re-

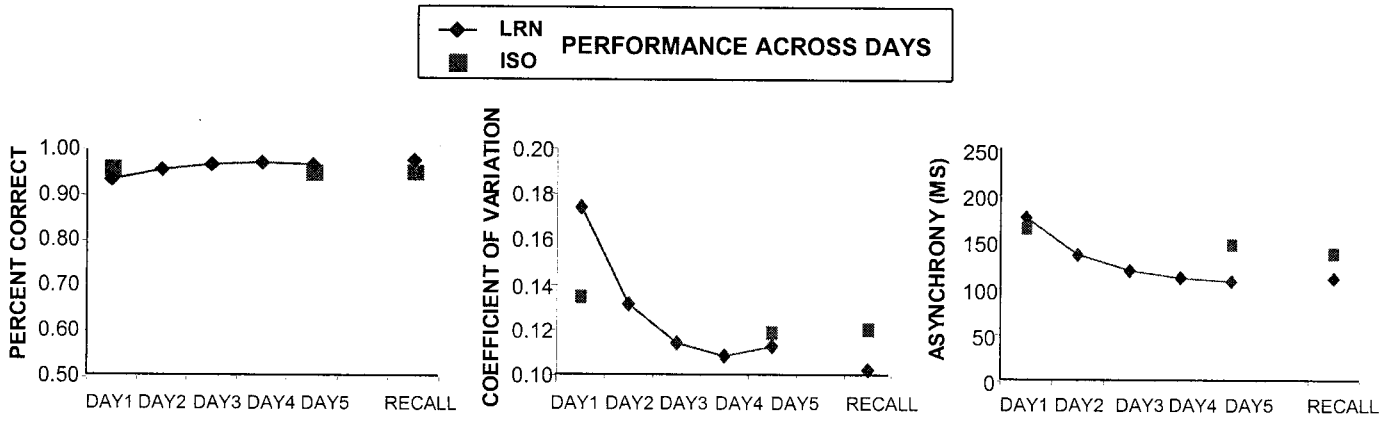

Figure 3. Illustrates changes in performance for the learned and isochronous sequences across days of practice (see Results). The *left graph* shows the change in percentage correct; the *middle graph* shows changes in the coefficient of variation, and the *right graph* shows changes in response asynchrony.

sponse durations. Response asynchrony was assessed by examining differences between stimulus onset and offset and the onset and offset of the subject's key-press responses. The variability and asynchrony measures were performed on correct responses only. All behavioral measures were averaged across blocks and days of practice. Differences across days 1–5 of practice, between day 5 and recall and across blocks of practice on day 1 were assessed using repeated measures ANOVA. In addition, comparison of these same measures was made between the LRN and the ISO conditions. Differences between LRN and ISO for each of the performance variables were assessed using ANOVA for repeated measures with significant interactions 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 sec scans, Siemens HR+, 3-D 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 305 template. PET volumes were normalized, reconstructed with a 12 mm Hanning filter, and averaged across subjects for each condition. Differences across days of learning were assessed using paired-image subtraction (Worsley et al., 1992), and by analyzing changes in normalized cerebral blood flow (nCBF) values from specific volumes of interest (VOI). For the subtraction analyses, statistically significant peaks were identified by an automatic algorithm with a threshold set at $t \geq \pm 3.5$. Activations identified as being in the same brain region that were located within 0.5 cm 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 were identified using a 3-D atlas of the human cerebellum in stereotaxic space (Schmahmann et al., 2000). For the nCBF analyses, spherical VOIs (radius, 5 mm) were defined using the Talairach locations of specific significantly active regions identified in the subtraction analyses. Average nCBF values for individual subjects were extracted for each VOI for both the LRN and ISO conditions on day 1, day 5, and recall. These values were submitted to repeated-measures ANOVA, and significant interactions were analyzed using tests of simple main effects with Bonferroni correction for multiple comparisons.

RESULTS

Behavioral data

No significant differences in overall performance were obtained for either the different tempos or the different sequences. Therefore behavioral data were collapsed across these dimensions. No significant change in simple percentage correct was observed across days 1–5 of learning (Fig. 3) (average day 1, 0.93; average day 5, 0.97; $F_{(4,32)} = 1.4$; $p = 0.25$) probably because each sequence of short and long elements was learned explicitly to criterion before scanning. However, significant changes were observed for both response variance (average day 1, 0.17; average day 5, 0.11; $F_{(4,32)} = 20.8$; $p < 0.001$) and response asynchrony (average day 1, 176 msec; average day 5, 108 msec; $F_{(4,32)} = 19.1$; $p < 0.001$). These results indicate that although the order of elements in the sequence was learned very rapidly, stabilization of response variance and synchronization continued to show significant effects of learning across days of practice. Importantly, no significant differences were obtained for any of the measures when comparing day 5 of learning to recall, indicating that once learned, both the sequence of elements and the temporal parameters were well retained (percentage correct: $F_{(1,8)} = 0.53$, $p = 0.49$; CV: $F_{(1,8)} = 2.6$, $p = 0.15$; asynchrony: $F_{(1,8)} = 0.38$, $p = 0.55$).

Behavioral measures for the learned sequences were also compared with those for the isochronous sequences on day 1, day 5, and recall. Results for percentage correct showed no significant change across days and no significant differences between the two conditions (day: $F_{(2,16)} = 0.25$, $p = 0.63$; condition: $F_{(1,8)} = 0.76$, $p = 0.48$; day × condition: $F_{(2,16)} = 0.43$, $p = 0.66$). For response variation, there was a significant day × condition interaction ($F_{(2,16)} = 5.3$; $p = 0.02$), such that the learned sequences showed significant change between day 1 and day 5 ($p = 0.004$), but the isochronous sequences did not ($p = 1.0$). Furthermore, the two conditions were significantly different on day 1 ($p < 0.001$), where the CV was lower for the isochronous sequences than for the learned sequences. This difference is probably the result of the simplicity of the isochronous sequences and the fact that they were always performed after the four blocks of practice on the learned condition. For the asynchrony measure, analysis revealed a significant effect of day ($F_{(2,16)} = 8.0$; $p < 0.01$), such that day 1 was significantly different than both day 5 ($p = 0.06$) and recall ($p = 0.01$). However, there was no significant effect of condition or any interaction, indicating that performance was similar across the two conditions. This is probably the result of general learning of the tapping response, irrespective of sequence complexity. Finally, behavioral data illustrating learning across blocks of practice on day 1 is shown in Figure 4 and will be considered further in the Discussion. Similar to the pattern of results across days of learning, there was no significant change in percentage correct across blocks

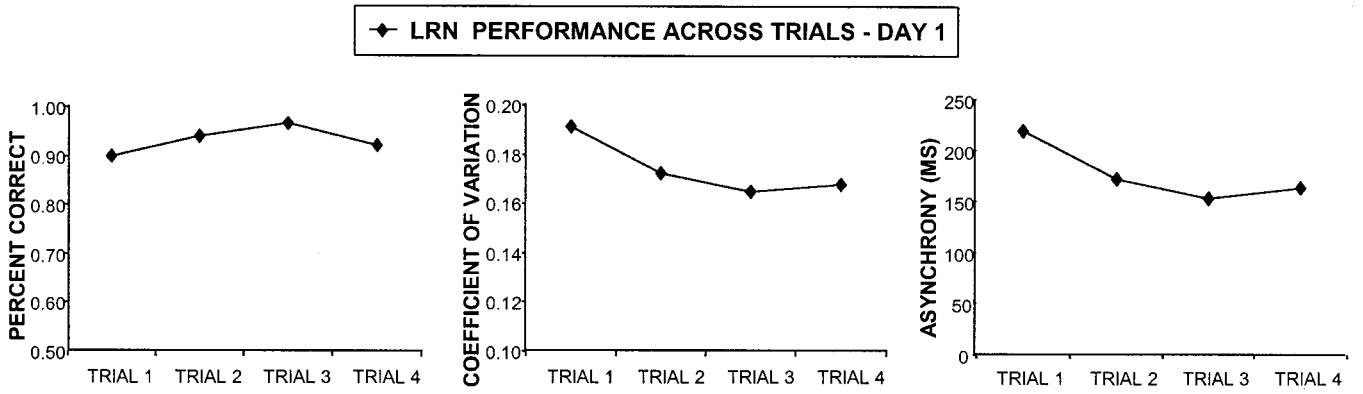

Figure 4. Illustrates changes in performance for the learned sequences across blocks of trials on day 1 of learning (see Discussion). The *left graph* shows the change in percentage correct, the *middle graph* shows changes in the coefficient of variation, and the *right graph* shows changes in response asynchrony.

Table 1. Locations of significant blood flow differences in the LRN1–ISO1 comparison

	Left				Right				Medial			
Location	*x*	*y*	*z*	*t*	*x*	*y*	*z*	*t*	*x*	*y*	*z*	*t*
Positive peaks												
Lobule III/IV[a]									−8	−46	−18	5.2
Lobule V/VI[a]									−6	−66	−18	4.5
Lobule VI lateral[a]	−28	−68	−22	3.7	20	−58	−28	3.5				
					42	−54	−26	4.9				
Lobule VIIIA[a]	−22	−62	−44	6.8	22	−66	−44	5.7				
					32	−52	−42	6.2				
					32	−54	−48	4.7				
Lobule VIIIB[a]	−26	−44	−42	7.0								
Lobule IX									2	−60	−44	4.8
Fusiform/parahippo					28	−42	−12	4.5				
Fusiform gyrus	−40	−50	−24	4.0								
Extra striate (18/19)									−10	−66	−4	3.7
Precuneus	−10	−68	24	3.9	20	−60	24	3.7				
Middle temporal gyrus					42	−8	−38	3.7				
					44	8	−36	3.6				
Uncus/parahippocampal	−26	−4	−38	3.6	22	−4	−38	3.5				
Pons									−8	−30	−30	3.5
Negative peaks												
Ventolateral frontal (47/11)	−40	42	−8	6.8	36	44	−4	6.2				
Superior frontal (6/8)	−22	16	54	5.3	28	12	52	4.7				
Lateral frontal (45)	−44	24	16	3.9								
Medial orbital frontal (14)									−4	48	−14	5.0
Medial frontal (8)									−2	38	46	4.9
Medial frontal (8)									−2	24	56	4.7
Middle temporal gyrus	−58	−46	−8	5.0	56	−38	−12	4.0				
Parietal (40)	−48	−58	42	4.8								

[a]Center location of VOI used in nCBF analyses.

1–4 on day 1 ($F_{(3,24)} = 1.52; p < 0.23$), but significant changes were observed for response variance ($F_{(3,24)} = 5.93; p < 0.01$) and response asynchrony ($F_{(3,24)} = 11.47; p < 0.01$).

Paired-image subtraction

LRN1 versus ISO1

Regions that were significantly more active during learning on day 1 were found in bilateral cerebellar cortex, extrastriate visual areas, and the hippocampal region. Active cerebellar regions included medial areas III/IV, V/VI, and IX. Activation in lateral regions were also seen bilaterally in lobules VI, VIIIA, and VIIIB (Table 1, Fig. 5). Because a similar number of movements were made in both conditions, activity in motor cortical regions that may have been involved in performing the sequences was not observed. Despite similar visual input, relatively greater activation was observed medially in areas 18/19 of extrastriate visual cortex and bilaterally in the precuneus and fusiform gyri. Increased blood flow in visual regions may be related to the sensorimotor integration demands of the task, requiring precise synchronization of the motor response with the visual stimulus (Bower, 1995).

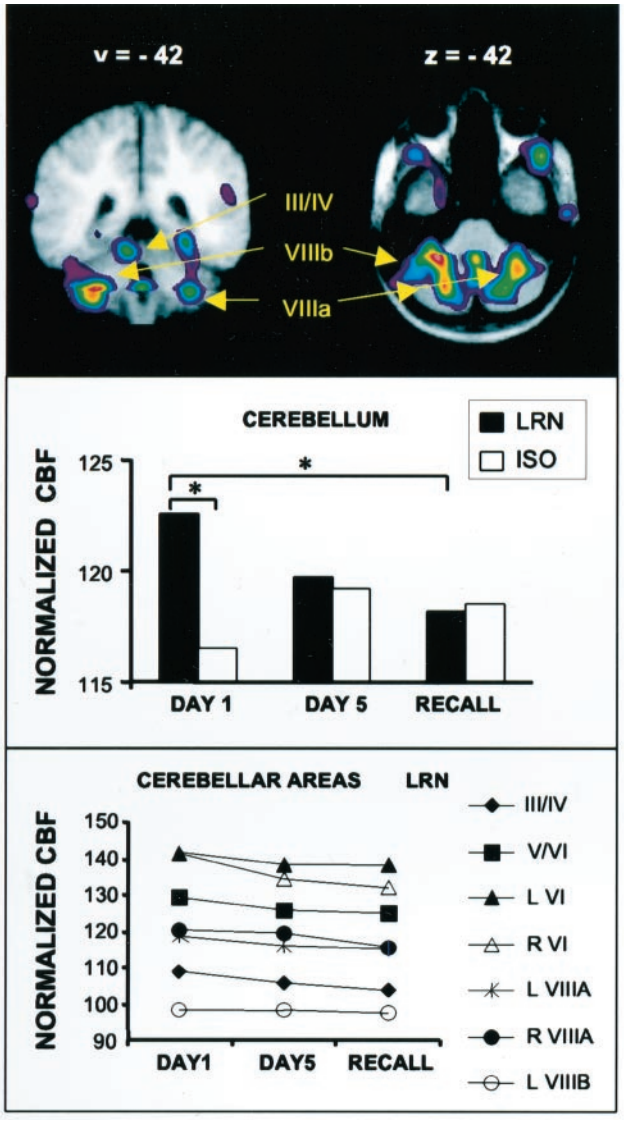

Figure 5. The *top panel* presents *z*-statistic maps showing significant regions of activation in the cerebellum on day 1 (LRN1–ISO1). PET data are coregistered with the average MRI of the nine subjects, and slice levels are given in the standardized space of Talairach and Tournoux (*t* value range, 2.5–7.0). The *bottom panel* shows graphs of the changes in nCBF values extracted from cerebellar VOIs for the LRN and ISO conditions. The *top graph* shows the significant decrease between day 1 and recall collapsed across all cerebellar regions (significant differences are indicated with an *asterisk*). The *bottom graph* shows changes in nCBF for the individual cerebellar regions for the LRN condition alone.

LRN2 versus LRN1

Comparison of LRN2 to LRN1 revealed a single area of residual activity in lobule IX of the cerebellum accompanied by relatively greater activity in the right putamen/globus pallidus (GP) and in the medial and orbital frontal cortex (Table 2, Fig. 6). Medial frontal activity included gyrus rectus (area 14 of Petrides), areas 9, 10, and 8 (Chiavaras and Petrides, 2000). The lateral orbital frontal gyri (areas 47/12 of Petrides) were also active bilaterally (Chiavaras and Petrides, 2000). Because cerebellar activity present in LRN2 could be masked in the comparison with LRN1, LRN2 was also compared with ISO2 (Table 3). The result of this comparison again revealed a single residual area of cerebellar activation, this time in lateral lobule VI/VIIa. These results are consistent with the hypothesis that the cerebellum is less actively involved in production of a motor sequence once it is well learned and that the BG and other cortical areas are more important during the more automatic phase of performance (Doyon and Ungerleider, 2002).

REC versus LRN2

When REC was compared with LRN2, relatively greater CBF was observed in left M1, PMC, inferior parietal cortex (area 40) and medial area 8 (Table 4, Fig. 7). No residual activity in the cerebellum or the BG was observed in either this comparison or in the REC versus ISO3 comparison (Table 5). These results are largely consistent with our working hypothesis and constitute the first demonstration that retention and production of a well learned motor sequence recruits a predominantly cortical network. Lack of cerebellar activation in these comparisons suggests that this structure is not required for the production of a well learned timed motor sequence, even after considerable delay.

CBF changes across days of learning

In order to directly examine blood flow changes across days of learning, nCBF values were analyzed for specific VOIs based on active regions identified in the subtraction analyses. VOIs were centered on the Talairach location of the highest *t* value for each region (for locations, see Tables 1, 2, and 4). Average nCBF values for each VOI were extracted from the LRN1, LRN2, and REC scans and for the ISO1, ISO2, and ISO3 scans. These values were submitted to a repeated measures ANOVA to examine changes in nCBF values across days of learning between the two conditions. Overall, analyses of the nCBF data confirmed the results of the subtraction analyses. Most importantly, they showed that nCBF changed significantly across days for the LRN, but not the ISO condition.

In the cerebellum, VOIs were created for the seven regions that were active in the LRN1–ISO1 subtraction but not the LRN2–LRN1 subtraction (Fig. 5, Table 1). Results showed a significant day × condition interaction ($F_{(2,16)} = 18.1$; $p < 0.001$), and tests of simple main effects showed that nCBF was greater in the LRN than the ISO condition on day 1. There was also a significant main effect of condition, such that nCBF was greater overall for the LRN than the ISO condition. Separate ANOVA for the LRN condition alone showed that nCBF decreased across all cerebellar regions across days ($F_{(2,16)} = 4.08$; $p < 0.04$). Tests of simple main effect revealed a marginally significant decrease for medial lobule III/IV between day 1 and recall ($p < 0.06$), and significant decreases between day 1 and day 5 ($p < 0.04$) and day 5 and recall ($p < 0.05$) for medial VI and right lateral lobule VI (day 1–day 5: $p < 0.01$; day 5–recall: $p < 0.001$). The difference between day 1 and day 5 was nearly significant for left lateral lobule VI ($p = 0.10$).

For the putamen/GP the VOI was centered on the peak of activation observed in the LRN2–LRN1 subtraction (Fig. 6, Table 2). Results of the ANOVA showed a significant day × condition interaction ($F_{(2,16)} = 5.9$; $p < 0.01$), with tests of simple main effect showing a marginally significant increase in nCBF for the LRN condition between day 1 and day 5 ($p = 0.06$), but no significant differences across days for the ISO condition ($p = 0.36$).

Changes in M1, PMC, the parietal lobe (area 40), and medial area 8 were examined for VOIs based on the peaks of activation observed in the REC–LRN2 subtraction (Fig. 7, Table 4). Results of an ANOVA including all four regions showed a significant day × condition interaction ($F_{(2,16)} = 12.4$; $p < 0.001$) such that

Table 2. Locations of significant blood flow differences in the LRN2–LRN1 comparison

	Left				Right				Medial			
Location	*x*	*y*	*z*	*t*	*x*	*y*	*z*	*t*	*x*	*y*	*z*	*t*
Positive peaks												
Medial orbital frontal (GR/14)									0	44	−16	8.4
Medial frontal polar (10)									−4	60	2	5.0
Medial superior frontal (9/10)									−10	58	28	4.2
Medial frontal (8)									−12	36	56	3.7
Ventrolateral frontal (47/12)	−42	38	−4	3.9	30	34	−12	4.3				
Lobule IX									−6	−48	−44	4.1
									−6	−58	−46	3.5
Putamen/GP[a]					28	−8	4	3.9				
Posterior cingulate									−4	−48	32	3.9
Middle temporal gyrus	−40	10	−38	3.7								
Negative peaks												
Lobule VI/VIIA					42	−54	−26	3.9				
Lobule VI (vermis)									−2	−74	−22	3.8
Lobule VIIIA	−22	−66	−48	3.6								
Angular gyrus					48	−42	4	4.1				
Precuneus					14	−72	48	3.5				
Cingulate (arm area)									−4	14	42	3.5
Parahippocampal gyrus					26	−22	−22	3.7				

[a]Center location of VOI used in nCBF analyses.

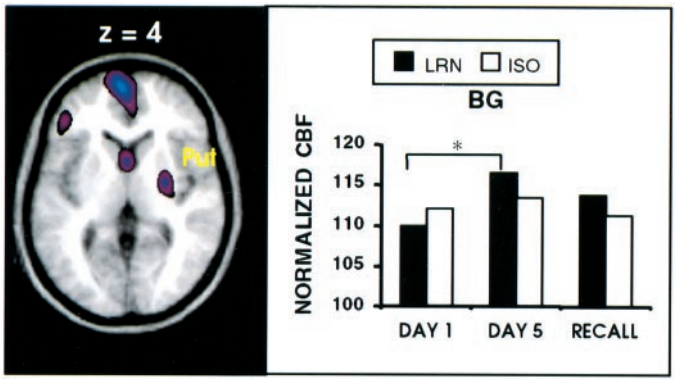

Figure 6. The *left panel* presents *z*-statistic maps showing the significant activation in the BG observed on day 5 (LRN2–LRN1). PET data are coregistered with the average MRI of the nine subjects, and slice levels are given in the standardized space of Talairach and Tournoux (*t* value range, 2.5–8.5). The *graph* illustrates changes in nCBF values extracted from the BG VOI for the LRN and ISO conditions. These results show a significant increase in activity between day 1 and day 5 (significant differences are indicated with an *asterisk*).

the LRN condition showed greater nCBF at recall than on day 5 ($p < 0.0001$) or day 1 ($p < 0.01$), but no differences were observed for the ISO condition. There was also a significant region × condition interaction, such that all regions differed for both conditions. Because of the large differences in overall nCBF between the regions, each region was submitted to a separate ANOVA. The results of these analyses showed the same overall pattern for M1 and PMC, with a significant day × condition interaction (M1: $F_{(2,16)} = 5.6$; $p < 0.01$; PMC: $F_{(2,16)} = 3.9$; $p < 0.04$) such that nCBF was greater at recall in comparison with day 5 (M1: $p < 0.001$; PMC: $p < 0.01$) and day 1 (M1: $p < 0.07$; PMC: $p < 0.01$). The day × condition interaction was also seen for the parietal lobe ($F_{(2,16)} = 3.5$; $p < 0.05$), with significant differences were found between day 1 and recall ($p < 0.04$). A marginally significant interaction was seen for medial area 8 ($F_{(2,16)} = 3.0$; $p < 0.08$), with a significant difference observed between day 1 and day 5 ($p < 0.02$). Taken together these results show that M1, PMC, and parietal cortex are more active during performance of the LRN sequences at recall than during performance of the same task as on day 5 of learning. This indicates that activity in these regions is specifically related to the delayed recall component of the task rather than any differences in task parameters.

DISCUSSION

These results demonstrate a network of cortical and subcortical structures that contribute differentially to the early and late phases of motor learning and to delayed recall. Early learning showed extensive activation of the cerebellar cortex. After 5 d of practice, cerebellar activity decreased and greater activity was observed in the BG and frontal lobe. At delayed recall, significantly greater activation was seen in M1, PMC, and the parietal lobe, with no significant activity in the cerebellum or BG. The results of the subtraction analyses were confirmed by changes in nCBF during learning compared with the isochronous baseline. Across days of learning, nCBF in the cerebellum decreased, but increased in the BG between day 1 and day 5. No significant changes were observed across days for the isochronous condition. At recall, nCBF for the learned sequences increased in M1, PMC, and parietal cortex, but not for the isochronous baseline. These findings support the working hypothesis that the cerebellum is primarily involved in the early phase of motor sequence learning, with the BG possibly contributing to a later, automatization phase. Importantly, this experiment demonstrates that relative to learning, delayed recall of a motor sequence appears to be mediated by a predominantly cortical network including M1, the PMC and parietal cortex.

Early learning

On day 1, greater activity was observed in cerebellar lobules III/IV and VI during performance of the LRN sequences than in

Table 3. Locations of significant blood flow differences in the LRN2–ISO2 comparison

	Left				Right				Medial			
Location	*x*	*y*	*z*	*t*	*x*	*y*	*z*	*t*	*x*	*y*	*z*	*t*
Positive peaks												
Medial orbital frontal (GR/14)									4	40	−16	5.5
Anterior cingulate (border 10)									0	48	−4	4.7
Lobule VI/VIIA					32	−66	−30	3.9				
Cuneus/border 17					10	−64	12	3.5				
Negative peaks												
Parietal (40)	−46	−46	46	5.1	48	−50	42	4.5				
Frontal polar (10)	−36	54	12	3.7								
Dorsolateral frontal (9)	−46	26	34	3.5								
Middle temporal sulcus					52	−36	−8	3.9				
Inferior temporal sulcus					32	6	−42	3.6				
Premotor (lateral 6)					32	10	52	3.5				

Table 4. Locations of significant blood flow differences in the REC–LRN2 comparison

	Left				Right				Medial			
Location	*x*	*y*	*z*	*t*	*x*	*y*	*z*	*t*	*x*	*y*	*z*	*t*
Positive peaks												
Premotor[a]	−20	−4	46	3.8								
M1[a]	−24	−22	58	3.7								
Parietal (40)[a]	−44	−32	44	3.5								
Medial frontal (8)[a]									−4	26	46	3.4
Negative peaks												
Anterior cingulate									4	30	−12	3.8
									6	36	6	3.5
									8	38	12	3.5
Cuneus									−12	−68	14	3.7
Fusiform gyrus					32	−40	−18	3.5				

[a]Center location of VOI used in nCBF analyses.

the ISO baseline. Greater cerebellar activation during initial performance of a motor task is consistent with a large number of recent studies (for review, see Doyon, 1997; Van Mier, 2000) (Doyon and Ungerleider, 2002). In addition, the specific cerebellar regions active on day 1 are similar to those observed in a previous study of performance of timed motor sequences (Penhune et al., 1998). Finally, these regions are consistent with those identified in a meta-analysis of cerebellar activity during motor sequence learning (Desmond and Fiez, 1998).

Several current theories describe specific cerebellar mechanisms that might mediate early learning: (1) combining of individual movements and motor context into movement "synergies" (Thach, 1996); (2) motor and perceptual timing (Ivry, 1996); and (3) sensorimotor integration (Bower, 1995) and error detection (Flament et al., 1996). Evidence that these mechanisms were active comes from changes in performance across the four blocks of learning on day 1 (Fig. 4). The percentage of correctly reproduced elements increased across blocks of practice, demonstrating improved performance of the motor sequence as a whole. Response variance and synchronization also improved, indicating refinement of movement timing and integration of the motor response with the visual stimulus. Participation of sensorimotor integration mechanisms in early learning is also supported by the observed activations in visual association areas in the LRN1–ISO1 subtraction. Extrastriate visual regions, predominantly in the dorsal stream, have strong connections to the cerebellum (Schmahmann, 1997). These visual association areas were not active in a previous study in which subjects imitated timed sequences after presentation of the stimuli (Penhune et al., 1998), further suggesting that activation in these regions is related to synchronization with the visual stimulus. Finally, error correction is an important component of early learning that encompasses the ability to modify responses in all of the above domains.

Late learning

A very different pattern of brain activity was observed after 5 d of practice, when task performance had stabilized (Fig. 3). Activity decreased in the cerebellum and increased in the BG and frontal cortex. Decreasing cerebellar activity as learning progresses is consistent with a number of previous studies (Grafton et al., 1994; Seitz et al., 1994; Toni et al., 1998; Doyon et al., 1999) and suggests that once a sequence is well learned, the timing and sensorimotor integration mechanisms active during early learning may not be called into play. Greater BG activity on day 5 is consistent with neuroimaging studies showing BG involvement in performance of well learned sequences (Doyon et al., 1996; Grafton et al., 1996; Rao et al., 1997; Rauch et al., 1997). BG involvement in the later phase of learning is also supported by

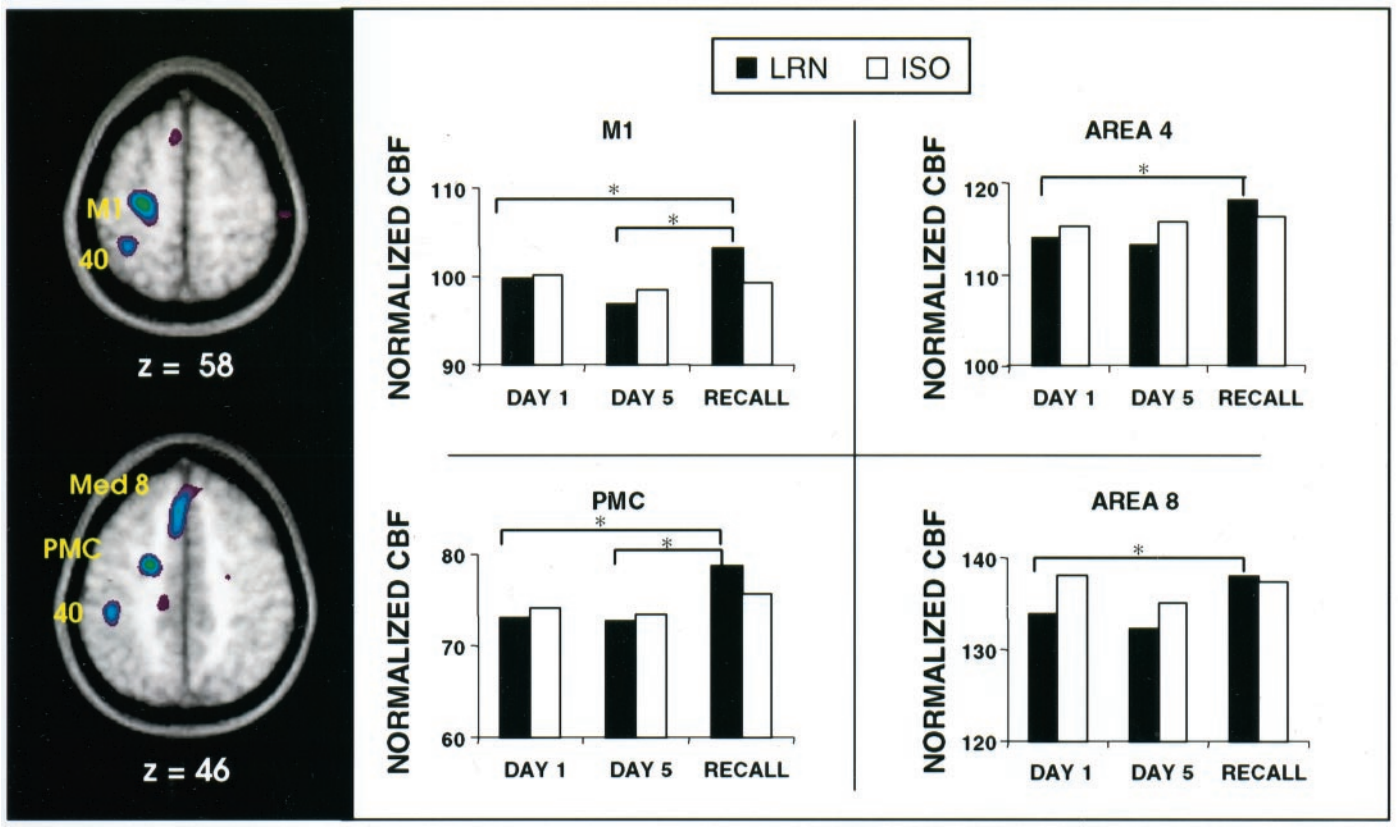

Figure 7. The *left panel* presents *z*-statistic maps showing significant regions of activation in M1, PMC, parietal cortex, and medial area 8 observed at recall (REC–LRN2). PET data are coregistered with the average MRI of the nine subjects, and slice levels are given in the standardized space of Talairach and Tournoux (*t* value range, 2.5–4.8). The *right panel* graphs changes in nCBF values extracted from each VOI for the LRN and ISO conditions (significant differences are indicated with an *asterisk*).

Table 5. Locations of significant blood flow differences in the REC–ISO3 comparison

	Left				Right				Medial			
Location	*x*	*y*	*z*	*t*	*x*	*y*	*z*	*t*	*x*	*y*	*z*	*t*
Positive peaks												
SMA (arm area)									0	−10	48	4
Lateral premotor	−24	−2	42	3.4								
Negative peaks												
Parietal (40)					48	−46	52	3.5				
Lateral premotor					20	12	64	3.5				

neurophysiological studies in animals (Graybiel, 1995) and by studies in Parkinson's disease showing impairments in late, but not early motor sequence acquisition (Doyon et al., 1997, 1998).

It has also been proposed that the BG are involved in motor and perceptual timing (Rao et al., 1997; Harrington et al., 1998). In the present experiment, timing mechanisms might be hypothesized to be maximally engaged for the LRN condition on day 1. However, no difference was seen between the LRN and ISO conditions, either in the subtraction or nCBF analyses. BG activity increased on day 5, when motor timing had become more accurate, as shown in both the LRN2–LRN1 subtraction and in the nCBF analysis. Therefore, BG appear to be most active when timing is well learned, suggesting a role in automatization for later recall. This interpretation is consistent with previous work showing greater BG activity during reproduction of simple timed sequences (Rao et al., 1997).

Finally, the BG are known to play a role in learning and memory for the motivational salience of responses (Schultz et al., 2000). Therefore, it might be possible that BG activity is the result of the rewarding properties of expert performance on day 5. However, no concomitant blood flow increase was observed in the BG during performance of the isochronous sequences, which were equally well performed.

On day 5, greater activity was also observed in ventrolateral and medial orbital frontal cortex. Ventrolateral frontal cortex has been shown to be involved in retrieval from short-term memory through connections with sensory association areas. (for review, see Petrides, 1994, 1995; Owen et al., 1996; Stern et al., 2000). Similar frontal regions were more active in ISO1 than in LRN1 (see negative peaks in Table 1), perhaps because these simple sequences could be learned quickly. Large increases in orbital frontal lobe activity were also observed on day 5. Two current

studies in our laboratory show activity in this region during performance of a well learned sequence of foot movements (Lafleur et al., 1999; Jackson et al., 2001). Additionally, medial orbital frontal cortex is implicated in reward (Elliott et al., 2000) and is strongly interconnected with the BG (Cavada et al., 2000). Activity in this region may reflect intrinsic reward associated with a high level of performance.

Delayed recall

Comparison of day 5 with recall revealed a different pattern of active regions, with significantly greater blood flow seen in left M1, PMC, parietal lobe (area 40), and medial area 8 (Fig. 7). nCBF analyses for M1, PMC, and the parietal lobe showed significant increases between day 5 and recall for the LRN, but not the ISO condition. Increased activity in M1 and PMC is consistent with neuroimaging studies of overlearned skills, such as typing and writing (Seitz et al., 1994; Gordon et al., 1998). Studies in humans and monkeys have shown changes in the degree or extent of activation in M1 related to long-term practice (Pascual-Leone et al., 1994; Karni et al., 1995; Nudo et al., 1996), and Karni has hypothesized that long-term representations of motor sequences may be stored in M1 (Karni et al., 1998). However, the few studies that have examined skill learning in humans with M1 damage have shown impairments in performance, but not in learning (Cushman and Caplan, 1987; Bondi et al., 1993; Platz et al., 1994; Winstein et al., 1999). Therefore, it seems unlikely that motor sequences are represented uniquely in M1, but are distributed within several motor cortical areas. The PMC, parietal cortex, and medial area 8 may form part of this network. The parietal cortex has been seen to be active during performance of overlearned sequences and may be involved in representation of somatosensory and body-centered spatial information (Sadato et al., 1996; Seitz et al., 1997; Sakai et al., 1998).

At recall, cerebellar activity decreased significantly compared with day 1, but was unchanged from day 5. This is consistent with data showing decreased cerebellar activity with learning (Grafton et al., 1994; Seitz et al., 1994; Toni et al., 1998; Doyon et al., 1999) and suggests that cerebellar mechanisms required during early learning are less engaged at recall. Unexpectedly, no significant BG activity was seen at recall, perhaps because of the relatively small number of subjects tested. Alternatively, however, this may be related to the explicit nature (Rauch et al., 1997) and simple motoric demands of the task.

This experiment reveals a dynamic network of cortical and subcortical structures active during early and late motor learning and at delayed recall. Based on these results, we propose that during early learning, the cerebellum is critically involved in adjusting movement kinematics according to sensory input to produce accurate motor output. During late learning, the BG may be involved in automatization of these parameters for delayed recall. At recall, cerebellar cortical mechanisms required for early learning do not appear to be called into play. At this phase, movement parameters may be encoded in a motor representation stored in a distributed network including M1, PMC, and parietal cortex.

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