

# fMRI investigation of cortical and subcortical networks in the learning of abstract and effector-specific representations of motor sequences

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A visuo-motor sequence can be learned as a series of visuo-spatial cues or as a sequence of effector movements. Earlier imaging studies have revealed that a network of brain areas is activated in the course of motor sequence learning. However, these studies do not address the question of the type of representation being established at various stages of visuo-motor sequence learning. In an earlier behavioral study, we demonstrated that acquisition of visuo-spatial sequence representation enables rapid learning in the early stage and progressive establishment of somato-motor representation helps speedier execution by the late stage. We conducted functional magnetic resonance imaging (fMRI) experiments wherein subjects learned and practiced the same sequence alternately in normal and rotated settings. In one rotated setting (visual), subjects learned a new motor sequence in response to an identical sequence of visual cues as in normal. In another rotated setting (motor), the display sequence was altered as compared to normal, but the same sequence of effector movements was used to perform the sequence. Comparison of different rotated settings revealed analogous transitions both in the cortical and subcortical sites during visuo-motor sequence learning—a transition of activity from parietal to parietal–premotor and then to premotor cortex and a concomitant shift was observed from anterior putamen to a combined activity in both anterior and posterior putamen and finally to posterior putamen. These results suggest a putative role for engagement of different cortical and subcortical networks at various stages of learning in supporting distinct sequence representations.

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## Introduction

It is commonly observed that when a skill is being acquired subjects are circumspect and deliberate in the initial attentive phase but later on as the skill is acquired they move into an automatic phase when attention can be engaged in other tasks simultaneously (Fitts, 1964). When performing well-mastered skills, it appears as if the body parts know what to do and no overt attention is necessary. Furthermore, the memory of over-learned skills seems robust and lasts for a long time. In this scenario, it is interesting to find out if the representation of skill memory and the associated neural bases are different at various stages of learning. Previous studies on sequence learning addressed where and when activity is found in various cortical and subcortical areas using implicit learning (Grafton et al., 1995) and explicit learning by trial and error (Sakai et al., 1998; Toni et al., 1998). This paper addresses the question of what is actually learned in different areas at different stages of explicit sequence learning.

Earlier studies that investigated representational changes during motor sequence learning emphasized either implicit sequence learning in the serial reaction time (SRT) paradigm (Grafton et al., 1998), explicit sequencing but without learning (Harrington et al., 2000), or the recall of motor sequences at various stages of learning (Karni et al., 1995; Penhune and Doyon, 2002). Grafton et al. (1998) found learning-related increases in regional cerebral blood flow (rCBF) in the sensorimotor cortex reflecting effector-specific representation and in the inferior parietal cortex reflecting abstract representation of motor sequences. Sakai et al. (1998) and Toni et al. (1998) used trial and error learning paradigm to study the time course of changes during explicit visuo-motor sequence learning.

Hikosaka et al. (2002) proposed that a sequence of movements is represented in two ways—spatial sequence and motor sequence. In their hypothetical scheme, spatial sequence learning and representation are supported by parietal–prefrontal cortical loops with the

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associative region of the basal ganglia (anterior striatum) and cerebellum (posterior cerebellum). Motor sequence learning and representation are mediated by the motor cortical loops with the motor region of the basal ganglia (posterior striatum) and cerebellum (anterior cerebellum). Furthermore, in their scheme, premotor area mediates the transformation of spatial to motor coordinates and presupplementary motor area (pre-SMA) participates in coordination or switching between the two representations. In this connection, it is interesting to note that this scheme is partly based on an earlier proposal of Alexander et al. (1986) of distinct cortico-basal ganglia-thalamus loops serving different functions. In this scheme that stresses parallel information processing, the dorsolateral prefrontal cortex (DLPFC)—caudate nucleus loop takes part in spatial sequencing whereas the supplementary motor area (SMA)—putamen loop mediates motor sequencing.

Our hypothesis was that motor sequence learning involves two representations—an early acquisition of effector-independent (abstract) representation and a late consolidation of effector-dependent representation (Hikosaka et al., 1999, 2002; Bapi et

al., 2000; Nakahara et al., 2001). In an earlier behavioral study (Bapi et al., 2000), we used a sequential button-pressing task in which subjects performed either the same visuo-spatial sequence with altered finger movements or a different visuo-spatial sequence with the same finger movements. We found that the response time was significantly shorter when the finger movements remained the same compared to when the visuo-spatial sequence was the same. These results suggest that an effector-independent representation develops early in the learning process and subsequently an effector-dependent sequence representation is formed.

Using a whole-brain fMRI study, we set out to investigate the question of the brain areas subserving such representations acquired at various stages of explicit learning of motor sequences. In the current study, subjects learned a sequence of 12 finger movements, using a 2 × 6 task (Fig. 1a) modified from Hikosaka et al. (1995), in two settings—normal setting where the visual display and keypad are arranged in the usual position and a rotated setting. In the rotated (motor and visual) conditions, subjects were required to rotate the visual cues by 180° and press the

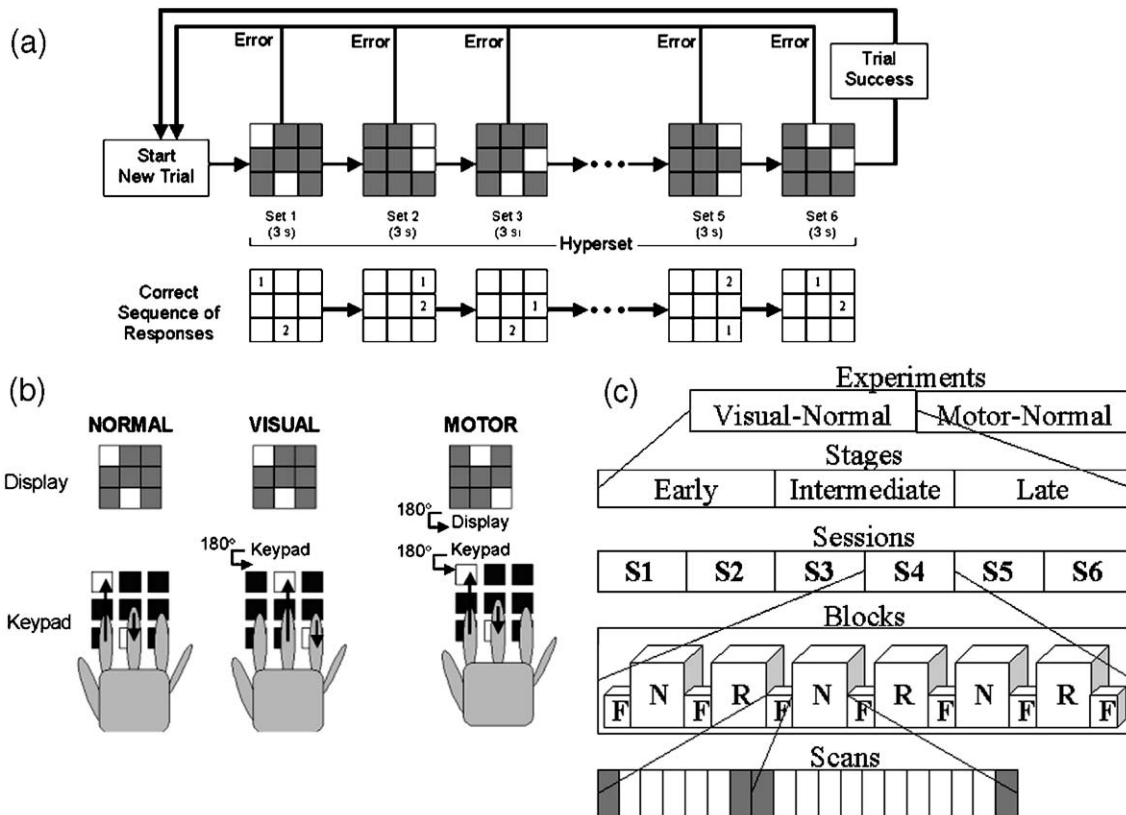


Fig. 1. Sequence task setup. (a) In 2 × 6 sequence task, a sequence of 12 key presses is learned by trial and error, two at a time (called a ‘set’) in a series of six sets (called a ‘hyperset’). The two key presses belonging to a set need to be executed within 3 s and after an appropriate delay the subsequent set is displayed. A new trial is started by resetting the presentation of the hyperset to the beginning either upon an error in any set or on successful completion of the entire hyperset. The bottom panel indicates the correct order of key presses for the example shown in the top panel. (b) Normal and Rotated settings for set 1 of the example are shown. In the normal setting, the visual display and keypad are arranged in the usual upright position and in the rotated settings the display-to-keypad relationship was altered. In the visual setting, the keypad was rotated by 180°, while the display remained unaltered. In the motor setting, both the keypad and the display on the screen were rotated by 180°. Consequently, in the visual setting, the sequence of visuo-spatial cues (visuo-spatial sequence) remained identical, while in the motor setting, the sequence of finger movements (somato-motor sequence) remained the same as that of the normal setting. Finger movements to be executed for an example are indicated by arrows in all the settings in the lower panel. Six such sets constituted a hyperset as shown in panel a. (c) Subjects performed two experiments—visual-normal and motor-normal. Each experiment consisted of six sessions of which the first two and the last two represented ‘early’ and ‘late’ stages of learning, respectively. We utilized an on–off (box-car) design for the experiments. In every session, subjects alternated between sequence learning tasks in the test blocks (N: normal and R: rotated) and followed random visual cues in the control blocks (F: follow). The duration of the test block was 36 s and that of control block was 21 s. 12 and 7 scans were acquired during the test and control blocks, respectively. Scans identified in gray shade represent instruction scans at the beginning and end of a block.

corresponding keys. The display sequence was also rotated for the motor condition, requiring an identical set of effector movements to be performed as in the normal condition. Thus, the display-to-keypad mapping was identical for both the motor and visual settings. Further in the visual setting, the sequence remained the same as in normal in visuo-spatial coordinates, whereas it was different from normal in somato-motor coordinates. On the other hand, in the motor setting, the sequence in somato-motor coordinates was the same as in normal, but it was different from normal in visuo-spatial coordinates. This experimental design allowed us to explicitly tap into the neural loci of abstract and effector-specific representations of motor sequences.

## Materials and methods

### Subjects

Ten normal (five female) right-handed subjects (ages 22–29 years) gave informed consent and were paid for their participation in the study. The experimental protocol was approved by the Ethics committee of the Laboratory for fMRI, Robarts Research Institute, London, Canada. Each subject contributed to two measurements by repeating the experiments on a different day. Subjects learned different sequences in the two repetitions of the experiments. Due to technical problems in data recording and large head movement, final data analysis was carried out on two repetitions by six subjects and one repetition by two other subjects.

### Experimental task

We used a modified  $2 \times 6$  sequence task (Hikosaka et al., 1995; Bapi et al., 2000). Two square cells (called a set) were illuminated simultaneously on the  $3 \times 3$  grid. Subjects learned, by trial and error, the correct order of pressing the corresponding keys. A sequence of six such sets constitutes a hyperset (Fig. 1a). Subjects were asked to execute the sequence as fast as they could at all times to facilitate smooth performance of finger movements. We fixed the inter-set gap within a hyperset to 3 s to enable presentation of the sets at an even pace. Trial was terminated upon an error and learning started again from the beginning of the hyperset.

### Normal and rotated settings

Subjects practiced the same hyperset alternately in normal and rotated (visual or motor) settings (Fig. 1b). While in the normal setting the display and keypad were arranged in the usual upright position, the display-to-keypad relationship was altered in the rotated settings. In the visual setting, the keypad was rotated by  $180^\circ$ , while the display remained unaltered. In the motor setting, both the keypad and the display on the screen were rotated by  $180^\circ$ . Consequently, in the visual setting, the sequence of visuo-spatial cues (visuo-spatial sequence) remained identical, while in the motor setting the sequence of finger movements (somato-motor sequence) remained the same as that of the normal setting. Furthermore, it is to be noted that the display-to-keypad mapping was identical between the motor and visual settings.

### Experiments

We utilized an on–off (box-car) design for the experiments where subjects alternated between control and test conditions (Fig.

1c). In the control condition, we used the  $1 \times 12$  follow task wherein subjects pressed one key at a time following random visual cues. In the test condition, we used the  $2 \times 6$  sequence task and subjects practiced the same hyperset alternately in normal and rotated (visual or motor) settings. Before every scanning experiment, subjects performed the experimental tasks in a practice session for half-an-hour using a hyperset different from the one used during scanning. Subjects were informed of the display and keypad rotations and hence our task is an explicit sequence learning task.

### Scan parameters

Scanning was done in a 4-Tesla Siemens/Varian MR whole-body imager at the laboratory for fMRI, Robarts Research Institute, University of Western Ontario, London, Canada. In each experiment, a time series of 726 whole-brain EPI images (interleaved Echo Planar pulse sequence) with an inter-scan interval of 3 s was acquired over a period of 6 sessions (Fig. 1c). Each scan consisted of 17 horizontal slices of 6 mm thickness [echo time (TE) 15 ms,  $64 \times 64$  matrix size,  $3.75 \times 3.75$  mm in-plane resolution, field of view (FOV) 24 cm]. In addition, a high-resolution anatomical image [Flash imaging sequence, TR 11 ms, TE 5.6 ms,  $256 \times 256$  matrix size,  $0.93 \times 0.93$  mm in-plane resolution] consisting of 64 slices separated by 3.3 mm, was collected for each subject.

### Data analysis

#### Behavioral analysis

While subjects performed experiments in the scanner, behavioral parameters reflecting the number of sets completed (accuracy) and the time taken to complete a set (response time) were measured for every block. Performance improvements within an experiment and across the normal and rotated settings were assessed using repeated-measures ANOVA. Based on the performance measures, we identified two learning periods—an early period comprising the first two sessions in an experiment where subjects were still slower and inaccurate and a late period comprising the last two sessions of an experiment where the subjects approached their maximal levels of performance.

#### Image analysis

The imaging data were analyzed using SPM99 (Wellcome Department of Cognitive Neurology, London). The functional images were reoriented to set the origin near the intersection of the coronal plane through AC and the AC–PC line and then motion correction was performed with respect to the first functional image in each session. Anatomical image for each subject was co-registered with the first functional image and then normalized to the T1 template from the International Consortium for Brain Mapping (ICBM) Project. The resulting parameters were used for normalizing all the functional images (Friston et al., 1995a) into Talairach stereotaxic space (Talairach and Tournoux, 1988). Spatial smoothing with a gaussian kernel of 8 mm FWHM was applied to the normalized images. The preprocessed data were analyzed using the general linear model framework (Friston et al., 1995b). For each subject, the experimental settings were modeled using boxcar functions convolved with the canonical hemodynamic response function in a session separable model. Data from all the six sessions were included. We report results from the early (first two sessions) and late stages (last two sessions) as the main focus of the

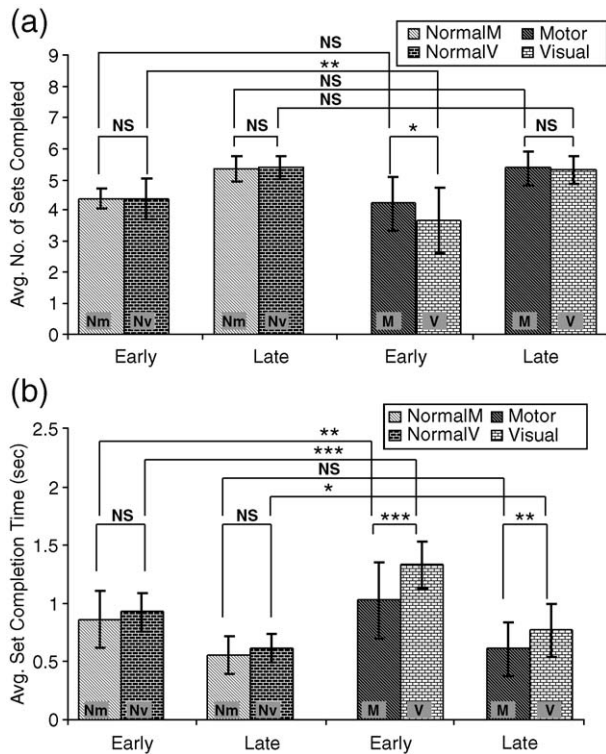


Fig. 2. Behavioral results. (a) Accuracy. Graph depicts the average number of sets completed (out of a maximum of 6 sets in a hyperset) in the early and late stages. As learning progressed, the number of sets completed (accuracy) increased significantly from early to late stage in all the settings ( $P < 0.0001$ ). Accuracy in the normal settings (NormalM and NormalV) was similar throughout the experiment. Although accuracy in the early stage seemed higher in the motor than in the visual setting, thereafter it remained similar in both the settings. (b) Set completion time. Graph depicts the average set completion time (in sec) in the early and late stages. As learning progressed, it required significantly less time to perform a set. Again, while the completion times were similar in the normal settings (NormalM and NormalV), they were much shorter for the motor than for the visual setting. Significance levels: \*\*\* $P < 0.0001$ , \*\* $P < 0.001$  and \* $P < 0.01$ , NS is not significant.

experiment was to examine representational changes in sequence learning. Furthermore, subject-specific variations in learning were taken into account by including the behavioral parameters (accuracy and response time) as user specified regressors. The regressors were constructed by giving a normalized score (range 0–100) reflecting improvements in learning for the sequence blocks. A score of 0 was assigned to the follow blocks as there was no learning involved.

Group analysis was performed using the random effects approach (Penny et al., 2003) as implemented in the SPM99 software. Contrast images computed from the subject-specific models were entered into paired  $t$  tests that accounted for the two repeated measures from the subjects. This model allowed for variance to be similar within subject and different across subjects. The voxel coordinates reported in the tables are transformed (Brett et al., 2001) from MNI to Talairach space. It was found that activated clusters spanned across several brain areas in the rotated > follow contrasts. For these contrasts, we counted the number of significant voxels using Talairach Daemon software (Lancaster et al., 2000). Location of peak activation for regions having more than 5

significant voxels was identified using ROI masks based on the Talairach daemon with the help of WFU Pick Atlas software (Maldjian et al., 2003). For the other contrasts, the number of significant voxels per cluster has been reported.

Brain activation results at selected cortical and subcortical areas are overlaid on normalized structural MRI of one of the subjects' skull stripped using the Brain Extraction Tool (Smith, 2002) available in the MRIcro software (Rorden and Brett, 2000). Functional overlays on transverse slices were achieved using MRIcro software. At selected functional regions of interest, average blood oxygen-level-dependent (BOLD) signal was calculated for each of the six sessions from a 3 mm spherical volume for each subject. We performed brain–behavior correlation analysis taking average values across subjects (Bland and Altman, 1994). Pearson correlation coefficient ( $R$ ) and its two-tailed significance level ( $P$ ) were computed.

## Results

### Behavioral results

Two behavioral measures were calculated—the average number of sets completed per trial in a block indicating the accuracy (Fig. 2a) and the average set completion time revealing the speed

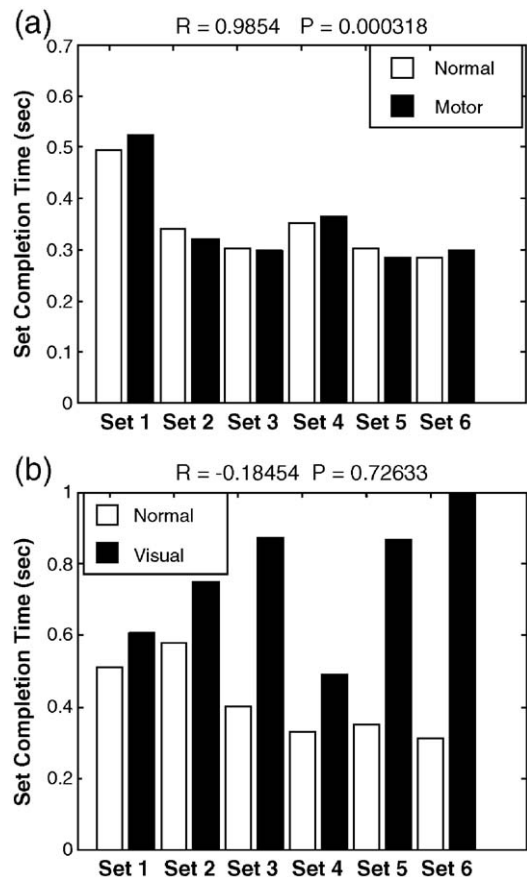


Fig. 3. Chunking results. Response time profile in motor setting seems to coincide with that of normalm indicating a transfer of motor skill from the normal to the motor setting. In contrast, the profile of RTs is distinctly different between normalv and visual settings pointing out that two motor sequence representations may have been acquired in the visual-normal experiments.

Table 1a  
Locations of significant BOLD signal in the early and late stages of visual > follow contrast

Brain area		Early					Late				
		Voxels	Coordinates (mm)			<i>t</i> value	Voxels	Coordinates (mm)			<i>t</i> value
			<i>x</i>	<i>y</i>	<i>z</i>			<i>x</i>	<i>y</i>	<i>z</i>	
Cerebellum (culmen)	R	105	2	-49	-9	4.13	111	8	-66	-8	6.5
Thalamus (pulvinar)	R	73	24	-29	3	4.48*	10	20	-24	16	3.34*
	L	55	-18	-23	14	5.96*	102	-20	-31	9	3.78
Caudate body	R	80	16	8	14	5.19	13	18	8	14	4.17
	L	21	-18	1	17	6.94	77	-20	-18	25	6.52
Anterior putamen (dorsal)	R	84	24	8	12	5.11*	27	20	3	15	4.89
	L	167	-24	4	3	5.84	128	-16	1	11	5.54
Posterior putamen (dorsal)	R	35	22	-1	15	5.75	58	30	-2	2	3.33*
	L	160	-30	-8	0	6.63	275	-28	-2	4	8.08
Globus pallidus	R	0	-	-	-	-	9	22	-14	-1	4.11*
	L	52	-16	0	9	4.62	83	-16	-1	9	4.84*
Ventral striatum	R	0	-	-	-	-	53	34	-20	-2	4.85
	L	98	-28	-6	-6	4.38*	68	-28	-4	-5	3.66*
Hippocampus	R	6	32	-33	-3	3.13*	21	32	-29	-7	4.08*
	L	0	-	-	-	-	0	-	-	-	-
Middle temporal gyrus (BA 39)	R	15	42	-71	26	4.54	25	48	-67	13	3.86
	L	2	-	-	-	-	0	-	-	-	-
Superior occipital gyrus (BA 19)	R	16	36	-74	28	4.16	16	38	-78	26	5.8
	L	8	-36	-78	32	4.5*	0	-	-	-	-
Middle occipital gyrus (BA 18/19)	R	3	-	-	-	-	10	28	-79	9	3.67
	L	17	-30	-79	19	4.09	14	-38	-72	7	3.45
Superior parietal cortex (BA 7)	R	102	32	-66	47	6.37	61	28	-46	58	6.05
	L	172	-24	-51	58	6.72	117	-24	-50	56	7.46
Inferior parietal lobule (BA 40)	R	97	42	-31	40	3.98	95	44	-33	48	5.93
	L	357	-40	-44	54	6.81	242	-36	-40	55	9.8
Precuneus (BA 7)	R	104	12	-55	58	4.73	69	10	-56	47	3.98
	L	108	-20	-56	53	8.77*	131	-12	-57	54	4.21*
Primary motor cortex (BA 4)	L	15	-40	-19	38	4.69*	22	-30	-27	46	4.98
Sensory (BA 2/3)	L	146	-55	-27	44	5.63	166	-55	-25	44	6.96
Premotor (dorsal) (BA 6)	R	0	-	-	-	-	7	-40	0	39	10.3
	L	40	-32	-6	41	4.68	46	32	6	42	6.41
Premotor (ventral) (BA 6)	R	0	-	-	-	-	0	-	-	-	-
	L	9	-36	0	35	5.59*	37	-48	-3	26	4.58
Pre-SMA (BA 6)		0	-	-	-	-	13	-2	14	45	3.8

Stereotaxic Talairach coordinates of peak activation obtained with  $P < 0.005$  (uncorrected). Coordinates of peak activation are reported for regions having at least 5 significant voxels. \* indicates that the peak was identified using a ROI mask as clusters of activations spanned across several brain regions.

of performance (Fig. 2b). Repeated measures ANOVA for the follow condition revealed that accuracy and response times were similar across the two experiments (visual-normal and motor-normal). Furthermore, there were no learning related improvements in the follow condition. Thus, follow serves as a stable baseline measure to assess the progress of performance in the test conditions.

Repeated measures ANOVA for the normal (*normalm*, *normalv*) and rotated (motor, visual) test conditions revealed significant ( $P < 0.0001$ ) improvements in accuracy and response times from the early to the late stage. Fig. 2 shows comparisons between the test conditions across the two experiments. While the normal conditions had similar performance measures, the comparisons between the rotated settings revealed superior performance

Table 1b  
Locations of significant BOLD signal in the early and late stages of visual > normal contrast

Brain area		Early				Late					
		Cluster size (voxels)	Coordinates (mm)			<i>t</i> value	Cluster size (voxels)	Coordinates (mm)			<i>t</i> value
			<i>x</i>	<i>y</i>	<i>z</i>			<i>x</i>	<i>y</i>	<i>z</i>	
Anterior putamen(d)	L		-	-	-	21	-26	2	7	4.57	
Superior occipital gyrus (BA 19)	R	21	34	-76	28	3.49		-	-	-	
Superior parietal cortex (BA 7)	R	43	34	-58	51	3.23		-	-	-	
	L	121	-16	-57	58	4.74		-	-	-	
Inferior parietal cortex (BA 40)	L		-	-	-	6	-44	-37	31	3.28	
Medial frontal gyrus (BA 9)			-	-	-	9	18	40	15	3.34	

Stereotaxic Talairach coordinates of peak activation obtained with  $P < 0.005$  (uncorrected).

for the motor setting than the visual setting. While subjects attained accuracy levels similar to that of the motor setting by the late stage of visual setting, the response times remained significantly slower in both early and late stages of visual setting.

To investigate the underlying causes for slower response times in the visual setting, we performed correlation analysis of chunking patterns between the normal and rotated settings. It has been shown that subjects spontaneously reorganize the sequence into a number of chunks while learning the *mxn* task (Sakai et al., 2003; Pammi et al., 2004). We identified the chunking patterns using the response times for individual sets of the sequence. We assumed that the chunking patterns would have stabilized by the late stage. Fig. 3 displays the average response times for the six sets in the late stage of normal and rotated settings for a representative subject. Since a trial was terminated upon error, only successful trials were used to calculate the chunking patterns. As shown in Fig. 3, there was a significant correlation between the chunking patterns of the normal and motor settings, while the chunking patterns were different for the normal and visual settings. The correlation results for all the subjects are tabulated in the supplementary material. These results clearly indicate that subjects used similar representation of motor sequences for the normal and motor settings, but developed a different representation of the motor sequence in the visual setting. Taken together, high accuracy level and slower response times in the visual setting in the late stage suggest that subjects might have successfully acquired a second motor sequence in the visual setting.

#### fMRI results

##### Visual-normal experiments

All the activations associated with visual-normal experiments during both the early and late stages are summarized in Table 1a and 1b and Fig. 4. In visual > follow contrast (Table 1a), sustained activation was found in the right cerebellum near the right superior vermis in the anterior lobe (culmen) in both early and late stages. Activations in other subcortical regions include the ventral striatum, caudate body, anterior and posterior regions of dorsal putamen, and right hippocampus. Activity in the left posterior putamen became stronger by the late stage. In addition, activation was also found in right middle temporal gyrus (BA 39) and extrastriate visual areas (BA 19). Activations in cortical regions include various regions of the parietal cortex, left primary motor cortex, and dorsal premotor cortex. Activation in the presupplementary motor area (pre-SMA) was observed in the late stage of visual setting. Activation in parietal regions was stronger in the left compared to the right hemisphere. The strength of activation in the left superior parietal cortex, left inferior parietal lobule (IPL), and left dorsal and ventral premotor areas showed an increasing trend from early to late stages. A transition of activation was observed from the parietal areas in the early stage to the parietal–premotor areas in the late stage of visual setting. In the visual > normal contrast (Table 1b), activity was found in the early stage in the superior occipital gyrus as well as in the superior parietal cortex. In the late stage, the activity was in the anterior putamen, inferior parietal cortex, and medial frontal gyrus.

##### Motor-normal experiments

All the activations associated with motor-normal experiments during both the early and late stages are summarized in Tables 2a and 2b and Fig. 5. In motor > follow contrast (Table 2a), there is a general trend of decrease in activation from early to late stage in various subcortical regions such as in the anterior lobe (culmen) of right

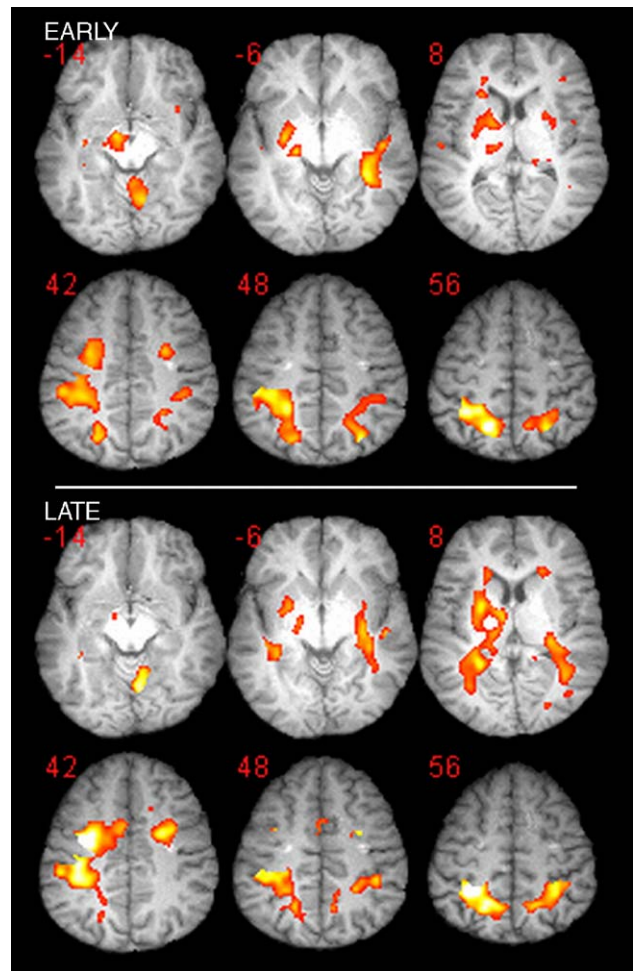


Fig. 4. Brain activation in the visual setting. Slice overlays depicting some of the subcortical and cortical activations found in the visual setting compared to the follow. Color scale indicates  $t$  values of activation thresholded at  $P < 0.005$  ranging from 0 to 7. Activations are overlaid on one of the subjects' skull-stripped normalized structural MRI. Top panel shows activations in early stage and bottom panel shows activations in late stage. The slices are selected to represent activations in the right anterior cerebellum ( $z = -14$  mm), ventral striatum ( $z = -6$  mm) and dorsal putamen ( $z = 8$  mm) in the top row and inferior parietal ( $z = 42$ ), dorsal premotor cortex ( $z = 42$ ), Pre-SMA ( $z = 48$ ) and Superior Parietal cortex ( $z = 56$ ) in the bottom row.

cerebellum, bilateral thalamus (pulvinar), right caudate body, bilateral dorsal putamen (anterior and posterior aspects), and bilateral globus pallidus. However, the trend in the cortical regions is a mixed one. Sustained activity was found in the left inferior parietal lobule, left primary motor cortex, and left somato-sensory cortex. While there is no residual activity in the right middle temporal gyrus and right middle occipital gyrus by the late stage, activity in the left middle occipital gyrus decreased by the late stage. Activation was found bilaterally in the superior parietal cortex, inferior parietal lobule, and precuneus in both early and late stages. An increase in activation was observed in both the dorsal and ventral regions of left premotor. While the activation in superior parietal cortex seems to decrease from early to the late stage, that in the dorsal premotor area in the left hemisphere becomes stronger by the late stage of motor setting. Thus, a transition of activation seems to be taking place from the parietal–premotor areas in the early stage to premotor areas by the late stage of motor setting. Interesting thing to

Table 2a  
Locations of significant BOLD signal in the early and late stages of motor > follow contrast

Brain area	Early					Late					
		Voxels	Coordinates (mm)			<i>t</i> value	Voxels	Coordinates (mm)			<i>t</i> value
			<i>x</i>	<i>y</i>	<i>z</i>			<i>x</i>	<i>y</i>	<i>z</i>	
Cerebellum (culmen)	R	130	10	−65	−9	5.88	26	6	−65	−9	4.2
Thalamus (pulvinar)	R	49	24	−29	5	4.94*	0		−		
	L	53	−4	−31	9	4.66	2		−		
Caudate body	R	30	10	5	13	3.55	7	14	6	7	4.6
	L	3		−			12	−16	16	14	3.81
Ant. putamen (dorsal)	R	44	26	8	11	4.48	3		−		
	L	10	−24	3	13	2.89*	1		−		
Posterior putamen (dorsal)	R	193	32	−9	6	3.86	5	24	−5	19	4.16
	L	178	−22	−3	15	5.05	45	−28	−4	6	3.62
Globus pallidus	R	27	22	−6	4	4.14	1		−		
	L	39	−20	−11	4	4.38*	0		−		
Middle temporal gyrus (BA 39)	R	10	42	−69	15	4.44	0		−		
	L	1		−			0		−		
Superior occipital gyrus (BA 19)	R	1		−			0		−		
	L	5	−36	−78	32	3.84*	0		−		
Middle occipital gyrus (BA 18/19)	R	16	28	−79	17	4.13	0		−		
	L	28	−28	−77	17	5.35	16	−26	−85	19	4.11
Superior parietal cortex (BA 7)	R	65	18	−47	61	4.05	47	24	−50	58	5.85
	L	76	−20	−49	60	7.5	98	−24	−56	53	5.89
Inferior parietal lobule (BA 40)	R	44	32	−38	50	3.52	23	36	−31	35	3.99
	L	262	−44	−36	52	4.28	289	−48	−33	40	6.21
Precuneus (BA 7)	R	79	20	−52	54	4.92*	9	20	−52	56	4.28*
	L	126	−20	−56	53	8.34*	64	−20	−54	54	4.98*
Primary motor cortex (BA 4)	L	13	−51	−16	34	4.01	17	−59	−19	40	4.27
Sensory (BA 2/3)	L	143	−44	−29	51	4.31	169	−48	−26	31	6.71
Premotor (dorsal) (BA 6)	R	4		−			12	28	4	40	7.47
	L	18	−30	−2	41	4.78	45	−32	4	40	7.81
Premotor (ventral) (BA 6)	R	2		−			0		−		
	L	13	−53	2	31	2.98	63	−42	−1	28	5.45
Middle frontal gyrus (BA 9/46)	R	14	44	8	35	3.82	0		−		
	L	0		−			26	−48	6	35	5.73

Stereotaxic Talairach coordinates of peak activation obtained with  $P < 0.005$  (uncorrected). Coordinates of peak activation are reported for regions having at least 5 significant voxels. \* indicates that the peak was identified using a ROI mask as clusters of activations spanned across several brain regions.

note is the absence of activity in the anterior dorsal striatum by the late stage and lack of any activity in the ventral striatum and the hippocampus in the motor setting. In motor > normal contrast, persistent activity was found in the left superior and inferior parietal cortical areas and in the early stage an additional locus of activation was found in the right middle temporal gyrus (Table 2b).

#### Distinct neural systems subserving sequence representations

Direct comparison of visual and motor contrasts would reveal distinct neural systems associated with the visual and motor settings, respectively (Table 3). In the early visual > motor comparison, left anterior putamen in the dorsal aspect, left ventral

striatum, and left hippocampus were activated. Apart from the subcortical activity, activation of the medial prefrontal cortex, anterior cingulate, right superior parietal cortex, and right occipital cortex near the middle occipital gyrus was also observed. Similarly, extensive cortical and subcortical activation was also observed in the late stage. Subcortical activation loci were found in the left dorsal putamen (anterior region) and left hippocampus. The activation in the left dorsal putamen was stronger in the late stage and extended into posterior putamen also. Cortical activation was in the precuneus, middle frontal gyrus, the occipital lobe, and the temporal lobe (BA 39). An interesting observation is the transition of activity observed from the left anterior putamen to

Table 2b  
Locations of significant BOLD signal in the early and late stages of motor>normal contrast

Brain area	Early				Late						
		Cluster size (voxels)	Coordinates (mm)			<i>t</i> value	Cluster size (voxels)	Coordinates (mm)			<i>t</i> value
			<i>x</i>	<i>y</i>	<i>z</i>			<i>x</i>	<i>y</i>	<i>z</i>	
Middle temporal gyrus (BA 39)	R	7	51	−63	25	3.24			−		
Superior parietal cortex (BA 7)	L	15	−21	−61	55	3.19	10	−34	−54	51	3.8
Inferior parietal lobule (BA 40)	L	7+4	−36	−42	46	3.3	23	−46	−37	39	3.63

Stereotaxic Talairach coordinates of peak activation obtained with  $P < 0.005$  (uncorrected).

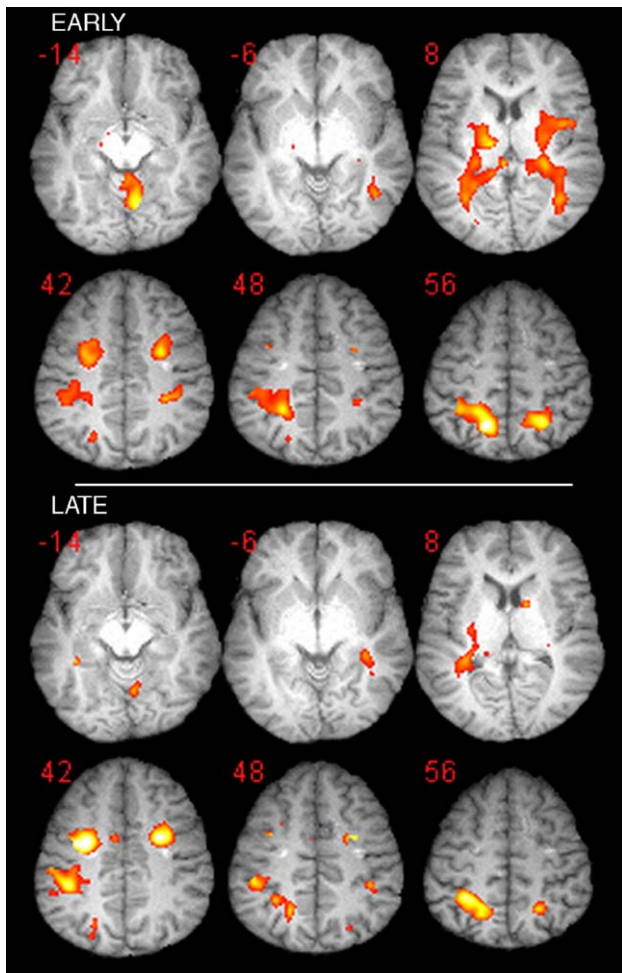


Fig. 5. Brain activation in the motor setting. Slice overlays depicting some of the subcortical and cortical activations observed in the motor setting compared to the follow. See Fig. 4 legend for further description.

left anterior and posterior putamen by the late stage in the visual setting (Table 3).

While the visual setting activated several subcortical and cortical sites, activity in the motor setting was restricted mainly to cortical loci. In the early motor > visual contrast, activation was observed in the precuneus, right occipital cortex near the lingual, fusiform and middle occipital gyri and in the right inferior frontal gyrus (IFG). While activation in the right IFG remained until the late stage, additional areas in the left prefrontal cortex (BA 9, 10 and 45), anterior cingulate, and caudal supplementary motor area (SMA) were activated in the late stage. There were activations in the right cerebellum (anterior lobe) and left caudate body in the late stage of the motor setting.

#### Summary of main effects and direct comparisons

While activation in the right anterior cerebellum was sustained from the early to late stage of visual setting, the activity decreased by the late stage in the motor setting. Whereas the activation in the left dorsal putamen extended into both anterior and posterior regions in the visual setting, that in the motor setting was found to be concentrated within the posterior region. Furthermore, activation in the putamen became stronger from early to late stage in the visual setting, but it decreased by the late stage in motor setting.

Activation in the ventral striatum and hippocampus was found only in the visual setting. There seems to be a trend of shift in activation from the parietal in early visual to parietal–premotor areas in late visual and early motor settings. The activation in premotor areas became stronger by the late stage of motor setting. The pre-SMA was active only in the late stage of visual setting and the caudal SMA only in the late stage of motor setting.

#### Brain–behavior correlation (BBC) analysis

In Table 4, we report the results from regression analysis of brain activation with the two behavioral parameters. Areas that have a positive correlation in visual setting would reflect sequence learning related changes while those with the motor setting would be related to performance of the somato-motor sequence. Correlation results depicted in Figs. 6 and 7 clearly establish the role for pre-SMA in the visual setting and that for the SMA in the motor setting. Correlation results indicate that as performance improved in both the rotated settings, the activity in the right DLPFC and the right superior parietal cortex decreased possibly reflecting processes related to motor sequence learning and performance (Jenkins et al., 1994; Jueptner et al., 1997a,b; Grafton et al., 1998). As performance improved in the visual setting, activity in pre-SMA, left ventral premotor increased and that in the left hippocampus decreased. On the other hand as performance became over-learned in the motor setting, activity in SMA and left dorsal premotor increased and that in the left inferior parietal cortex and precuneus decreased.

#### Discussion

The aim of the study was to investigate different representations of visuo-motor sequences as learning progressed from the early to the late stage. We demonstrated that response times were shorter when subjects used effector-specific information (as in the motor setting) than when they utilized abstract sequential information based on visual cues (in the visual setting). We identified the subcortical and cortical brain areas that mediated the two sequence representations. Firstly, left anterior putamen in the dorsal striatum was found to be selectively active in the visual setting. Secondly, it appears that when sequences are learned utilizing visuo-spatial representation, the focus of activation moved from parietal in the early stage to parietal–premotor areas in the late stage. In contrast, when sequences were performed with an emphasis on the somato-motor representation, the transition was in the opposite direction, that is, from parietal–premotor in the early to premotor areas in the late stage.

It is known that visuo-spatial sequence representation can be acquired quite quickly but somato-motor optimization takes time (Bapi et al., 2000; Nakahara et al., 2001). In the visual setting, although subjects attained similar level of accuracy to that of motor setting by the late stage, performance speed was significantly slower (Fig. 2). Behavioral results further indicated that the subjects utilized abstract sequential information provided by the visual cues over an extended period of time and eventually learned a new somato-motor sequence in the visual setting. Superior performance speed in the motor setting by the early stage itself (Fig. 2) underlines the advantage of using effector-specific representation. We further demonstrated that chunking patterns of the sequences were identical between the motor and normal settings (Fig. 3). Thus, while the onset of learning of visuo-spatial

Table 3  
Locations of significant BOLD signal comparing the two rotated conditions

Brain area		Cluster size (voxels)	Coordinates (mm)			<i>t</i> value
			<i>x</i>	<i>y</i>	<i>z</i>	
<i>Early visual &gt; motor</i>						
Ventral striatum	L	3 + 3	–32	–12	–8	2.93
Anterior putamen (dorsal)	L	3	–26	4	2	2.96
Hippocampus	L	11	–30	–22	–11	3.27
Brodman area 19	R	23	36	–41	–3	3.47
	L	5	–26	–68	–3	2.94
Medial frontal gyrus (BA 9) +		155	–4	38	29	3.85
Anterior cingulate (BA 32)		155	–6	30	22	3.47
<i>Late visual &gt; motor</i>						
Caudate body	L	19 + 24	–18	–18	23	3.59
Medial globus pallidus	L	6	–18	–14	–4	3.06
Hippocampus	L	3	–28	–35	0	3.04
Anterior putamen (dorsal)	L	95	–30	4	3	4.08
Thalamus (Pulvinar)	L	4	–14	–23	7	2.98
Middle occipital gyrus (BA 19)	R	158	22	–89	8	4.66
Inferior parietal lobule (BA 40)	L	21	–28	–38	53	3.33
Superior parietal cortex (BA 7)	R	6	14	–55	58	3.12
Precuneus (BA 7)	R	41	8	–54	47	3.71
Middle frontal gyrus (BA 10)	R	10	34	38	18	3.69
<i>Early motor &gt; visual</i>						
Brodman area 19	R	42	28	–81	17	3.97
Brodman area 18	R	15	30	–78	–3	3.56
	L	11	–6	–69	15	3.43
Cingulate gyrus (BA 31)	R	96	16	–53	28	3.7
	L	9	–16	–45	28	3.02
Precuneus (BA 7)	L	165	–16	–70	37	4.53
Inferior frontal gyrus (BA 45)	R	3	55	20	5	3.12
<i>Late motor &gt; visual</i>						
Anterior cerebellum (culmen)	R	9	12	–34	–10	3.07
Caudate body	L	3	–8	5	16	3.17
Anterior cingulate (BA 32)	L	6	–6	41	7	3.06
SMA (BA 6)		7	0	–25	53	3.22
Superior frontal gyrus (BA 10)	R	4	–28	48	23	3.18
Middle frontal gyrus (BA 9)	L	11	–44	11	33	3.53
Inferior frontal gyrus (BA 45)	R	3	57	26	15	3.15
	L	12	–53	22	8	3.37
Medial frontal gyrus		5	–8	53	16	3.46

Stereotaxic Talairach coordinates of peak activation obtained with  $P < 0.005$  (uncorrected).

and somato-motor sequence representations is the same, the activity associated with each setting at various stages pointed out how learning of the representations unfolded over time. The unique experimental design we used in this study enabled us to tap into the representations that facilitate the learning of motor sequences and their unfolding process.

#### Subcortical structures

While activation in the right anterior cerebellum sustained from the early to late stage of visual setting (Table 1a), the activity decreased by the late stage in the motor setting (Table 2a). Anterior cerebellum might possibly be involved in the optimization of movement parameter and timing information in both the sequence representations (Jueptner and Weiller, 1998; Sakai et al., 2000). Based on previous studies involving trial and error learning (Jenkins et al., 1994; Jueptner et al., 1997b), sustained activity

observed in the ventral striatum in both early and late stages of visual setting may be attributed to the trial and error process adopted for cue selection. Interestingly, when there was no emphasis on cue selection process as in the motor setting, ventral striatal activity was absent. Further evidence comes from the activation of ventral striatum observed in the direct comparison contrast of early visual > motor (Table 3). Left putamen activation in the dorsal striatum extended into both anterior and posterior regions in the visual setting (Table 1a), whereas in the motor setting activation was found exclusively within the posterior putamen (Table 2a). Furthermore, left anterior putamen in the dorsal striatum was found to be selectively active in the visual setting (Table 3). Additionally, while activation in the putamen became stronger from the early to late stage in the visual setting, it decreased by the late stage in the motor setting (Tables 1a and 2a). Another structure that can possibly be implicated with visuo-spatial representation is the hippocampus, which was found to be

Table 4  
Brain–behavior correlations in the rotated settings

Brain area	Coordinates (mm)			Visual				Motor				
				Correlation (accuracy)		Correlation (response time)		Correlation (accuracy)		Correlation (response time)		
	<i>x</i>	<i>y</i>	<i>z</i>	R	P	R	P	R	P	R	P	
Ant. cerebellum (culmen)	R	10	−65	−9	+0.80	0.058	−0.76	0.08	+0.34	0.51	−0.37	0.47
Hippocampus	L	−22	−35	−10	−0.96	<0.01	+0.96	<0.01	+0.25	0.63	−0.22	0.68
Ant. putamen (d)	L	−26	6	5	+0.78	0.067	−0.74	0.94	+0.077	0.89	−0.087	0.87
Post. putamen (d)	L	−26	−9	10	+0.81	<0.05	−0.78	0.06	+0.13	0.81	−0.11	0.84
Post. putamen (d)	L	−26	−2	0	+0.41	0.41	−0.41	0.42	+0.77	0.07	−0.77	0.07
Sup. parietal cortex (BA 7)	R	34	−58	51	−0.85	<0.05	+0.89	<0.05	−0.82	<0.05	+0.81	0.052
Inf. parietal lobe (BA 40)	L	−34	−54	40	−0.65	0.16	+0.72	0.11	−0.85	<0.05	+0.87	<0.05
Precuneus (BA 7)	R	4	−58	51	−0.29	0.58	+0.36	0.48	−0.94	<0.01	+0.93	<0.01
	L	−6	−63	51	−0.49	0.32	+0.57	0.23	−0.88	<0.05	+0.87	<0.05
Premotor (dorsal) (BA 6)	L	−40	−2	41	+0.37	0.47	−0.33	0.52	+0.81	<0.05	−0.85	<0.05
Premotor (ventral) (BA 6)	L	−55	1	26	+0.98	<0.001	−0.97	<0.001	−0.08	0.87	+0.14	0.79
Pre-SMA (BA 6)		−4	8	46	+0.99	<0.0001	−0.99	<0.0001	+0.39	0.44	−0.38	0.46
SMA (BA 6)		−6	−17	49	+0.18	0.73	−0.16	0.75	+0.87	<0.05	−0.86	<0.05
Middle frontal gyrus (BA 46)	R	30	19	34	−0.81	0.051	+0.84	<0.05	−0.84	<0.05	+0.84	<0.05

Correlation of brain activation with behavioral measures. Abbreviations: Sup., Superior; d, Dorsal; Ant., Anterior; Post., Posterior.

activated in early and late stages of visual > motor contrast (Table 3). The brain–behavior relationship in the hippocampus was significantly correlated with the visual setting, but not the motor setting (Table 4). In contrast to earlier studies where cerebellum (Doyon et al., 2003) and basal ganglia (Penhune and Doyon, 2002; Shadmehr and Holcomb, 1997) activity was not observed during the recall stage, the activity in these regions persisted until the more automatic late stage in the motor setting. One possible reason for this is that, in our experiment, recall was measured within the experimental session on the same day as opposed to the earlier studies where recall was assessed after a delayed consolidation period. The conclusions on cerebellum are provisional as the coverage of cerebellum did not extend into posterior lobe in the fMRI scans at the chosen field of view.

#### Cortical structures

There seems to be a trend of shift in activation from the parietal in early visual to parietal–premotor areas in late visual (Table 1a) and early motor settings (Table 2a). The activation in premotor areas becomes stronger by the late stage of motor setting (Table 2a). The trend of shift in parietal areas is strengthened by its activation in the late visual > motor comparison and early motor > visual contrasts (Table 3). The brain–behavior relationships clearly demonstrate a decreasing trend of activation in the parietal areas and an increasing trend of activation in the premotor areas for the motor setting (Table 4). The activation in the frontal areas did not show any selectivity to either of the rotated settings (Tables 3 and 4). The rostral part of supplementary motor area, pre-SMA, was selectively active in the late stage of visual setting (Table 2a). In the visual-normal experiments, subjects used the same visuo-spatial sequence but learned two motor sequences, one corresponding to the normal and the other to the visual setting. The brain–behavioral relationship result of pre-SMA (Fig. 6, Table

4) combined with the selective activation found in the late but not in the early stage of visual setting (see Table 1a) indicates its role in new somato-motor sequence learning process. The activation of pre-SMA during new motor sequence learning was also observed in earlier studies (Hikosaka et al., 1996; Sakai et al., 1998). Together with the activity observed in the anterior cingulate (Table 3), we speculate that the pre-SMA may have a dual role, one in sequence learning and the other in sequence switching (Shima et al., 1996). Consistent with the explicit and implicit sequence learning literature (Tanji and Shima, 1994; Grafton et al., 1998; Willingham, 1998; Keele et al., 2003; Tanji, 2001), we found SMA activity in the late stage of motor setting (Table 3) and brain–behavior correlation (Fig. 7, Table 4) reflecting its role in somato-motor sequence representation. We observed activity in M1 during somato-motor sequence learning and performance as evidenced by its activation in early and late stages of both the rotated settings. These results suggest that M1 participates in the learning of motor sequences but may not be the actual locus of representation. These observations on M1 are consistent with most of the earlier proposals for its role in motor learning (for example Karni et al., 1995; Grafton et al., 1998; Sanes, 2003). We can speculate that activity in the right DLPFC observed in both the rotated settings (decreasing trend as seen in Table 4) is related to the optimization of the sequencing process.

It is possible that the rotated > normal contrasts point out learning-related activations in addition to rotational transformations. Our experimental design involved interleaved blocks of normal and rotated settings alternating with the follow condition. Both the normal and rotated settings involved sequence learning, whereas the follow condition did not involve any learning. Hence, we associate any activity observed in the subtractions reflected in rotated > normal contrasts with rotational transformations, but not with sequence learning. Activity observed in the superior occipital gyrus and superior parietal cortex in the visual > normal contrast

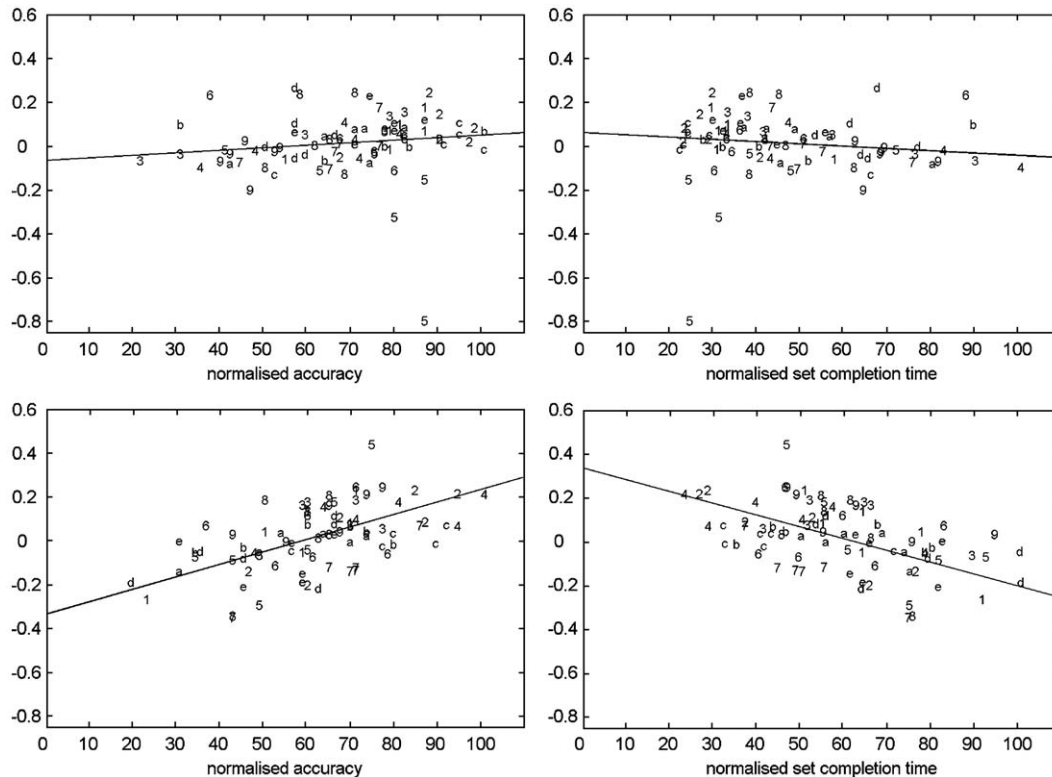


Fig. 6. Brain–behavior correlation at Pre-SMA. Voxel data were extracted from a 3 mm spherical VOI defined at Pre-SMA (Talairach coordinates  $x = -4$ ,  $y = 8$ ,  $z = 46$  mm; Table 3) and the average BOLD signal for each of the six sessions was calculated for each subject separately for motor and visual experiments. Average performance measures were calculated for the six sessions for each subject separately for the two experiments. Graph depicts the scatter plot between the normalized behavioral measure (accuracy: left panel and set completion time: right panel) and the BOLD signal for each subject (1–9 and a–e). The regression line fits the average values of behavioral measure and the BOLD signal. The top panel shows the results for motor setting and the bottom panel for the visual setting. The correlation results are: Motor [Accuracy:  $R = 0.39$ ,  $P = 0.44$ ; Time:  $R = -0.38$ ,  $P = 0.46$ ]; Visual [Accuracy:  $R = 0.99$ ,  $P < 0.0001$ ; Time:  $R = -0.99$ ,  $P < 0.0001$ ]. Clearly, the activity in pre-SMA is closely related to performance improvements in the visual setting but not the motor setting.

(Table 1b) may support processes involved in target rotation and the activity in the middle temporal gyrus and inferior parietal cortex in the motor > normal contrast (Table 2b) may be related to the processes involved in cue rotation.

#### *Brain areas supporting the formation of representations during sequence learning*

Our results suggest that visuo-spatial sequence representation engages cortical and subcortical network involving the left anterior striatum, hippocampus, extrastriate visual areas, dorsal and ventral premotor, and parietal cortical areas (see Tables 1a, and 3). The activation in the extrastriate visual areas may be related to the visuo-motor processes required for synchronizing the motor actions to visual cues (Bower, 1995). The activation in the extrastriate visual areas and hippocampus may form part of the ventral stream (Mishkin et al., 1983) that encodes information in visual coordinates and conveys the information to ventral premotor to enable the formation of visual stimulus-to-response associations (Caminiti et al., 1998). The activity in the parietal cortex may be part of the dorsal stream (Mishkin et al., 1983) conveying information in spatial coordinates to the dorsal premotor to enable formation of spatial cue-to-response associations (Wise et al., 1997). Anterior striatum may be in the best position to combine the information from the ventral and dorsal streams to formulate goal-directed action sequences based on abstract information. Our

results also revealed that effector-specific sequence representation is subserved by the dorsal premotor and SMA (Tables 3 and 4). A summary of our findings on various representations acquired during the process of visuo-motor sequence learning is given in the supplementary figure (Fig. S4).

#### *Cortico-subcortical networks subserving visuo-spatial and somato-motor sequence representations*

Our hypothesis in sequence learning is that the early stage involves abstract (visuo-spatial) representation and the late stage involves effector-specific (somato-motor) representation. This is true regardless of whether subjects are performing the sequence task in the normal, motor, or visual settings in our experiments or they are learning some other visuo-motor skill such as cycling or boxing. The usual acquisition of visuo-spatial sequence being very rapid, we designed the visual-normal experiment so that subjects were required to use the sequence of visual cues over an extended period and eventually acquired a second motor sequence for the visual setting. The design strategy of the motor-normal experiments was such that subjects could focus on motor movement sequence right from the early stage and progress to performance of an over-learned somato-motor sequence. Ours is an explicit sequence learning task and the instructions and pretraining given to the subjects ensured that subjects took advantage of the representations that were being learned at various stages of the

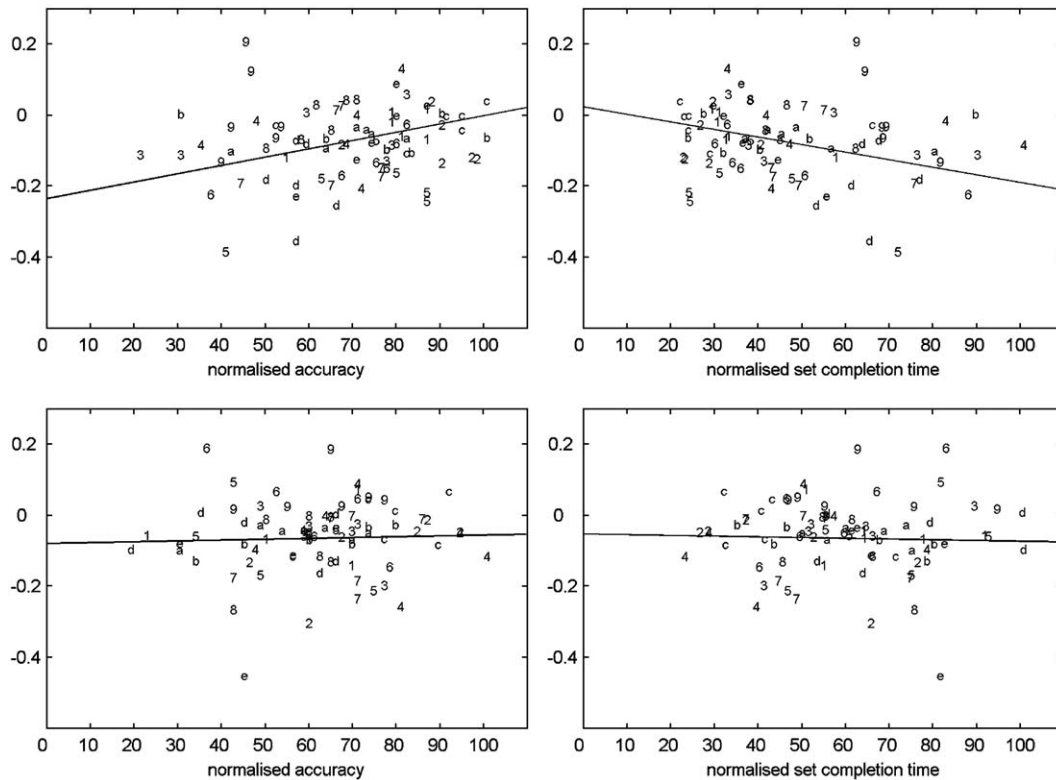


Fig. 7. Brain–behavior correlation at SMA. The correlation analysis procedure is described in the caption for Fig. 6. Voxel data were extracted from a VOI defined at SMA (Talairach coordinates  $x = -6$ ,  $y = -17$ ,  $z = 49$  mm; Table 4a). The top panel shows the results for motor setting and the bottom panel for the visual setting. The correlation results are: Motor [Accuracy:  $R = 0.87$ ,  $P < 0.05$ ; Time:  $R = -0.86$ ,  $P < 0.05$ ]; Visual [Accuracy:  $R = 0.18$ ,  $P = 0.73$ ; Time:  $R = -0.16$ ,  $P = 0.75$ ]. Clearly, the activity in SMA is closely related to performance improvements in the motor setting but not the visual setting.

task (see Experiments). However, it is possible that there are alternative explanations for the results we obtained.

First, subjects might simply be performing the display and keypad rotations to retrieve the sequence from the normal setting in order to perform the rotated settings. This is not likely given that the accuracy was similar for the motor and normal settings in both early and late stages. The visual setting had accuracy similar to normal by late stage (Fig. 2). Further if subjects had continued to use rotational transformations until the late stage, we would not observe improvements in response times from the early to the late stage.

Second, subjects might develop novel representations for the sequence in rotated settings independently from the sequence in the normal setting. This is not likely given that subjects are slower in the visual setting compared to the motor setting in both the early and late stages. We have manipulated the display and keypad rotations such that display-to-keypad mapping is the same in visual and motor settings. If subjects did not benefit from the sequence in the normal setting, then improvements in performance must be similar for the two rotated settings and that is clearly not the case (Fig. 2).

Third, it is possible that in both the rotated settings, subjects simply learned to apply rotational transformations in the initial phase to learn the correct sequence but eventually learned to replace this difficult operation with a novel sequence representation. We suggest that this is a more plausible explanation for sequence learning tasks that require performing a rotational transformation such as the current experiment. Given that the visuo-spatial representation is acquired fairly quickly during

sequence learning (for example, in the normal setting), the visual setting stretches this representation over a longer period of time as evidenced by its slower performance indices. Similarly, given that a somato-motor representation takes quite long to establish, the motor setting extends this late stage of sequence learning into the early stage of the motor setting by using strategies of explicit sequence learning. Thus, the tasks appear to bias the sequence learning process in the two experiments the way we intended.

The shift in activity from anterior putamen in early visual (Table 1a and 3) to posterior putamen in late visual (Table 1a) and early motor settings and finally to the posterior putamen in the late stage of motor setting (Table 2a) seems to have a cortical analog. The transition of activity from the parietal cortex in the early visual setting to conjoint activity in the parietal–premotor areas in the late visual as well as early motor and subsequently to the dominant activity in the premotor cortex in the late stage of motor setting seems to mirror the type of transitions taking place in the basal ganglia regions. Anterior striatum is part of the prefrontal and parietal cortex–basal ganglia loops which may be involved in the visuo-spatial representation whereas posterior striatum as part of the primary and secondary motor cortex–basal ganglia loops may be involved in the somato-motor sequence representation (Alexander et al., 1986; Hikosaka et al., 2002).

The novelty of our current findings is that the differential involvement of the cortico-subcortical loops subserving various sequence representations could be demonstrated as a direct outcome of our experimental design. The results suggest that during the process of learning, an early acquisition of visuo-spatial representation is subserved by frontal, parietal cortex–

anterior striatal loop followed by an additional recruitment of secondary motor areas (dorsal premotor cortex and SMA)—posterior striatal loop during the acquisition of somato-motor representation. Finally, these results form the first comprehensive and direct evidence for the model proposed by Nakahara et al. (2001). The current results additionally suggest that possible reason for activation of different cortical and subcortical networks at various stages of sequence learning is to support two kinds of representation—abstract (visuo-spatial) in the early stage and effector-specific (somato-motor) in the late stage.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2006.04.205.

### References

- Alexander, G.E., DeLong, M.R., Strick, P.L., 1986. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu. Rev. Neurosci.* 9, 357–381.
- Bapi, R.S., Doya, K., Harner, A.M., 2000. Evidence for effector independent and effector dependent representations and their differential time course of acquisition during motor sequence learning. *Exp. Brain Res.* 132, 149–162.
- Bland, J.M., Altman, D.G., 1994. Correlation, regression, and repeated data. *BMJ* 308, 896.
- Bower, J., 1995. The cerebellum as a sensory acquisition controller. *Hum. Brain Mapp.* 2, 255–256.
- Brett, M., Christoff, K., Cusack, R., Lancaster, J., 2001. Using the Talairach atlas with the MNI template. *NeuroImage* 13, S85.
- Caminiti, R., Ferraina, S., Mayer, A.B., 1998. Visuomotor transformations: early cortical mechanisms of reaching. *Curr. Opin. Neurobiol.* 8, 753–761.
- Doyon, J., Penhune, V., Ungerleider, L.G., 2003. Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning. *Neuropsychologia* 41 (3), 252–262.
- Fitts, P.M., 1964. Perceptual-motor skill learning. In: Melton, A.W. (Ed.), *Categories of Human Learning*. Academic Press, New York, pp. 243–285.
- Friston, K.J., Ashburner, J., Frith, C.D., Poline, J-B., Heather, J.D., Frackowiak, R.S., 1995. Spatial registration and normalisation of images. *Hum. Brain Mapp.* 2, 165–189.
- Friston, K.J., Holmes, A.P., Worsley, K.J., Poline, J-B., Frith, C.D., Frackowiak, R.S., 1995. Statistical parametric maps in functional imaging: a general linear approach. *Hum. Brain Mapp.* 2, 189–210.
- Grafton, S.T., Hazeltine, E., Ivry, R., 1995. Functional mapping of sequence learning in normal humans. *J. Cogn. Neurosci.* 7, 497–510.
- Grafton, S.T., Hazeltine, E., Ivry, R.B., 1998. Abstract and effector-specific representations of motor sequences identified with PET. *J. Neurosci.* 18, 9420–9428.
- Harrington, D.L., Rao, S.M., Haaland, K.Y., Bobholz, J.A., Mayer, A.R., Binder, J.R., Cox, R.W., 2000. Specialized neural systems underlying representations of sequential movements. *J. Cogn. Neurosci.* 12, 56–77.
- Hikosaka, O., Rand, M.K., Miyachi, S., Miyashita, K., 1995. Learning of sequential movements in the monkey: process of learning and retention of memory. *J. Neurophysiol.* 74, 1652–1661.
- Hikosaka, O., Sakai, K., Miyauchi, S., Takino, R., Sasaki, Y., Putz, B., 1996. Activation of human presupplementary motor area in learning of sequential procedures: a functional MRI study. *J. Neurophysiol.* 76, 617–621.
- Hikosaka, O., Nakahara, H., Rand, M.K., Sakai, K., Lu, X., Nakamura, K., Miyachi, S., Doya, K., 1999. Parallel neural networks for learning sequential procedures. *Trends Neurosci.* 22, 464–471.
- Hikosaka, O., Nakamura, K., Sakai, K., Nakahara, H., 2002. Central mechanisms of motor skill learning. *Curr. Opin. Neurobiol.* 12, 217–222.
- Jenkins, I.H., Brooks, D.J., Nixon, P.D., Frackowiak, R.S., Passingham, R.E., 1994. Motor sequence learning: a study with positron emission tomography. *J. Neurosci.* 14, 3775–3790.
- Jueptner, M., Weiller, C., 1998. A review of differences between basal ganglia and cerebellar control of movements as revealed by functional imaging studies. *Brain* 121, 1437–1449.
- Jueptner, M., Stephan, K.M., Frith, C.D., Brooks, D.J., Frackowiak, R.S.J., Passingham, R.E., 1997. Anatomy of motor learning: I. Frontal cortex and attention to action. *J. Neurophysiol.* 77, 1313–1324.
- Jueptner, M., Frith, C.D., Brooks, D.J., Frackowiak, R.S.J., Passingham, R.E., 1997. Anatomy of motor learning: II. Subcortical structures and learning by trial and error. *J. Neurophysiol.* 77, 1325–1337.
- Karni, A., Meyer, G., Jezzard, P., Adams, M.M., Turner, R., Ungerleider, L.G., 1995. Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 377, 155–158.
- Keele, S.W., Ivry, R., Mayr, U., Hazeltine, E., 2003. The cognitive and neural architecture of sequence representation. *Psychol. Rev.* 110 (2), 316–339.
- Lancaster, J.L., Woldorff, M.G., Parsons, L.M., Liotti, M., Freitas, C.S., Rainey, L., Kochunov, P.V., Nickerson, D., Mikiten, S.A., Fox, P.T., 2000. Automated Talairach atlas labels for functional brain mapping. *Hum. Brain Mapp.* 10, 120–131.
- Maldjian, J.A., Laurienti, P.J., Kraft, R.A., Burdette, J.H., 2003. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fmri data sets. *NeuroImage* 19, 1233–1239.
- Mishkin, M., Ungerleider, L.G., Macko, K.A., 1983. Object vision and spatial vision: two cortical pathways. *Trends Neurosci.* 6, 414–417.
- Nakahara, H., Doya, K., Hikosaka, O., 2001. Parallel cortico basal ganglia mechanisms for acquisition and execution of visuomotor sequences—A computational approach. *J. Cogn. Neurosci.* 13, 626–647.
- Pammi, V.S.C., Miyapuram, K.P., Bapi, R.S., Doya, K., 2004. Chunking phenomenon in complex sequential skill learning in humans. In: Pal, N.R., Kasabov, N., Mudi, R.K., Pal, S., Parui, S.K. (Eds.), *Lecture Notes in Computer Science*, 3316. Springer-Verlag Heidelberg, pp. 294–299.
- Penhune, V.B., Doyon, J., 2002. Dynamic cortical and subcortical networks in learning and delayed recall of timed motor sequences. *J. Neurosci.* 22 (4), 1397–1406.
- Penny, W.D., Holmes, A.P., Friston, K.J., 2003. Random effects analysis. In: Frackowiak, R.S.J., Friston, K.J., Frith, C., Dolan, R., Friston, K.J., Price, C.J., Zeki, S., Ashburner, J., Penny, W.D. (Eds.), *Human Brain Function*, 2nd ed. Academic Press.
- Rorden, C., Brett, M., 2000. Stereotaxic display of brain lesions. *Behav. Neurol.* 12 (4), 191–200.
- Sakai, K., Hikosaka, O., Miyauchi, S., Takino, R., Sasaki, Y., Putz, B., 1998. Transition of brain activation from frontal to parietal areas in visuomotor sequence learning. *J. Neurosci.* 18, 1827–1840.
- Sakai, K., Hikosaka, O., Takino, R., Miyauchi, S., Nielsen, M., Tamada, T.,

2000. What and when—Parallel and convergent processing in motor control. *J. Neurosci.* 20, 2691–2700.
- Sakai, K., Kitaguchi, K., Hikosaka, O., 2003. Chunking during human visuomotor sequence learning. *Exp. Brain Res.* 132, 149–162.
- Sanes, J.N., 2003. Neocortical mechanisms in motor learning. *Curr. Opin. Neurobiol.* 13, 225–231.
- Shadmehr, R., Holcomb, H.H., 1997. Neural correlates of motor memory consolidation. *Science* 277, 821–825.
- Shima, K., Mushiake, H., Saito, N., Tanji, J., 1996. Role for cells in the presupplementary motor area in updating motor plans. *Proc. Nat. Acad. Sci. U. S. A.* 93 (16), 8694–8698.
- Smith, S.M., 2002. Fast robust automated brain extraction. *Hum. Brain Mapp.* 17 (3), 143–155.
- Talairach, J., Tournoux, P., 1988. *Co-Planar Stereotaxic Atlas of the Human Brain*. Thieme, New York.
- Tanji, J., 2001. Sequential organization of multiple movements: involvement of cortical motor areas. *Annu. Rev. Neurosci.* 24, 631–651.
- Tanji, J., Shima, K., 1994. Role of supplementary motor areas cells in planning several movements ahead. *Nature* 371, 413–416.
- Toni, I., Krams, M., Turner, R., Passingham, R.E., 1998. The time course of changes during motor sequence learning: a whole-brain fMRI study. *NeuroImage* 8, 50–61.
- Willingham, D.B., 1998. A neuropsychological theory of motor skill learning. *Psychol. Rev.* 105, 558–584.
- Wise, S.P., Boussaoud, D., Johnson, P.B., Caminiti, R., 1997. Premotor and parietal cortex: corticocortical connectivity and combinatorial computations. *Annu. Rev. Neurosci.* 20, 25–42.