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The functional neuroanatomy of simple and complex sequential finger movements: a PET study

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Summary

The brain regions activated by simple repetitive and sequential finger movements of different length were localized by measuring regional cerebral blood flow (rCBF) with PET. The experimental design consisted of finger movements cued by auditory pacing at 0.5 Hz. In all conditions of different sequence length the contralateral primary sensorimotor and premotor cortex, supplementary motor area and ipsilateral cerebellar cortex were activated. These areas showed a large increase in activation from rest to simple repetitive movement, and a further increase with the shortest sequence, suggesting an executive role in running sequences. The ipsilateral premotor area (Brodmann area 6), bilateral posterior parietal areas (Brodmann area 7) and precuneus showed an increase in rCBF related only to the length of the sequences, without any change from rest to simple repetitive movement. These areas are more selectively related to sequence performance. This finding is consistent with the hypothesis that these areas function in the storage of motor sequences in spatial working memory. Our results suggest that sequential finger movements recruit discrete sets of brain areas with different functions.

Keywords: sequential movement; simple movement; premotor cortex; parietal cortex; PET

Abbreviations: FWHM = full width at half-maximum; PMC = premotor cortex; rCBF = regional cerebral blood flow; SM1 = primary sensorimotor cortex; SMA = supplementary motor area; TMS = transcranial magnetic stimulation

Introduction

Mechanisms underlying the execution of simple and complex motor tasks have drawn the attention of many investigators. The contralateral primary sensorimotor cortex (SM1) is always activated with voluntary movement, and surely plays an executive role. The supplementary motor area (SMA) is also frequently activated during the execution of movements, but in addition is activated with movement ideation, and its precise role is not clear (Orgogozo and Larsen, 1979; Deiber *et al*., 1996). The premotor cortex (PMC) is activated by motor tasks involving the generation of sequences from memory (Halsband *et al*., 1993), motor learning (Jenkins *et al*., 1994) and selection of movement (Deiber *et al*., 1991).

Performance of a motor sequence requires the execution of preprogrammed temporal and spatial movement patterns. Many reports concern the regions related to the complexity of movement (Orgogozo and Larsen, 1979; Roland *et al*., 1980*b*; Grafton *et al*., 1992; Halsband *et al*., 1993; Rao *et al*., 1993; Shibasaki *et al*., 1993). There is evidence that both the SMA and the PMC have an important role in the generation of sequences from memory that fit into a precise timing plan. Lesions of the SMA and PMC resulted in impairment of programming of sequential and rhythmic patterns from memory (Halsband *et al*., 1993). Shibasaki *et al*. (1993) in a PET study found a greater increase in regional cerebral blood flow (rCBF) in the ipsilateral SM1 and the SMA during self-paced complex finger movements than during simple, simultaneous movement of all the fingers. Other rCBF studies, however, have not disclosed any difference between the execution of simple and complex movements (Roland *et al*., 1980*a*; Colebatch *et al*., 1991). The heterogeneity of these results might result, at least in part, from the nature of the motor tasks used in different studies (Remy *et al*., 1994).

In a recent study from our group on sequential movements (Sadato *et al*., 1996*a*), in which an increase in complexity was related to the length of the sequence, activation occurred

in the contralateral SM1, ventral premotor, posterior SMA and ipsilateral cerebellum with the shortest finger sequence, but activation in these regions did not continue to increase as sequence length increased. On the other hand, a linear increase in rCBF in the right dorsal premotor and right precuneus was related to increasing sequence length. Simple movements were not included in the study design. However, since activation in SM1 areas may be saturated at the rate used in this study (2 Hz; Sadato *et al*., 1996*b*), the question of changes in SM1 activation with increasing length of sequence may have been confounded.

Kawashima *et al*. (1994) reported that the M1 hand area contains subregions that are related to preparatory activity and subregions that change their activity with the learning of new motor skills. Such findings challenge the traditional concepts of the simple executive role of the M1. Karni *et al*. (1995), using functional MRI, and Pascual-Leone (1995), using single-pulse transcranial magnetic stimulation (TMS) to map motor representations of hand muscles, found changes in M1 related to learning of movement sequences. A more recent study from our group using TMS (Corwell *et al*., 1996) suggests that M1 may be critically involved in processing complex sequences.

The purpose of the present study was to determine the brain regions activated in association with motor sequences of different lengths performed at rates that preclude saturation in activation. We included simple movement in the study design, and we tried to differentiate the brain areas involved in motor execution of simple and sequential movements and brain areas associated with longer sequential tasks, which presumably correspond to different functions. The older age of the subjects and the slow rate of motor performance make the results of the study appropriate for comparisons with those of patients.

Methods

Subjects

We studied 13 normal volunteers (eight male, five female), aged 41–64 years (mean 51.7 years), who had no history of neurological disease and no abnormalities on physical and neurological examinations. The subjects were all right-handed by the Edinburgh Inventory (Oldfield, 1971). The protocol was approved by the Institutional Review Board, and all subjects gave their written informed consent for the study.

Experimental design

The experimental paradigm consisted of five different conditions: four conditions of sequential right finger tapping with different lengths of the unit sequence as an index of complexity (Table 1) and one rest condition as control. The shortest sequence involved just repetitive flexion movement of the right index finger against the thumb; therefore it is referred to as 'simple movement'. Three sequences of variable length of unit sequences involved all right fingers in their execution. They are referred to as 'sequential conditions' or individually, with respect to the number of movements in each condition, as 'sequence-4', 'sequence-12' and 'sequence-16'. For the movement tasks, subjects briskly and precisely touched the tip of the thumb with the fingers of the right hand at a frequency of 0.5 Hz, paced to the beat of a metronome. The subjects were instructed to wait for the tone and move after that. The finger movements were monitored by an electrically equipped glove, which recorded the timing and the finger that tapped the thumb. Performance of the sequence was assessed by calculating the percentage of correct taps. No omission of taps was observed. Before PET scanning all subjects practised the sequences until they could perform them from memory 10 times in a row without errors. At this level of performance the sequences were considered 'overlearned', thus assuring constant performance during the experimental session at an approximately similar level of training.

Table 1 *Sequences of opponent finger movements*

Task	Unit sequence	Length of unit sequence
Simple	1	1
Sequence-4	1,2,3,4	4
Sequence-12	1,2,3,4,1,3,2,4,4,2,3,1	12
Sequence-16	1,2,3,4,1,3,2,4,4,2,3,1,4,3,2,1	16

Unit sequence: 1, index finger; 2, middle finger; 3, ring finger; 4, little finger.

Each subject underwent six consecutive scans at 12-min intervals, one for each of the five conditions and one additional condition of 'free-chosen' movement, which is not reported here. For the rest scan, subjects lay quietly listening to the 0.5 Hz beat of the metronome. No attempt was made to control the subject's thought content or attention during rest. For the movement scans, the subjects started finger movements when the metronome began sounding, which was simultaneous with the time of the radioisotope injection, and moved constantly until the end of the scan. The order of the different movement tasks and rest scans was randomised across all subjects to avoid an order effect.

PET procedure

The PET scans were performed with a Scanditronix PC 2048–15B (Uppsala, Sweden), which collected 15 contiguous planes with an in-plane resolution of 6.5 mm full width at half-maximum (FWHM) after reconstruction, and with a centre-to-centre distance of 6.5 mm, covering 97.5 mm in the axial direction. Each slice was 6.5 mm thick. Field of view and pixel size of the reconstructed images were 256 and 2 mm, respectively. A transmission scan was obtained with a rotating $^{68}Ge/^{68}Ga$ source. Based on the reconstructed transmission images, the position of the head was set to cover SMA, sacrificing views of the inferior part of the

cerebellum. The subjects lay comfortably in a supine position with their eyes covered for the duration of the experiment. A small plastic catheter was placed in the left cubital vein for injection of the radioisotope. The subject's head was immobilized with an individually fitted, rigid thermoplastic face mask that was attached to the scanner bed.

Reconstructed images were obtained by summing the activity during the 60-s period following the first detection of an increase in cerebral radioactivity after the intravenous bolus injection of 50 mCi of ^{15}O water. No arterial blood sampling was performed, and thus the images collected were those of tissue activity. Tissue activity recorded by this method has been shown to be linearly related to rCBF (Fox *et al*., 1984; Fox and Mintun, 1989).

Image analysis

Data analysis was performed with statistical parametric mapping (using SPM '95 from the Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (Mathworks, Natick, Mass., USA). Statistical parametric mapping combines the general linear model (to create the statistical map, or SPM) and the theory of Gaussian fields to make statistical inferences about regional effects (Friston *et al*., 1991; Worsley *et al*., 1992; Friston *et al*., 1994).

The scans from each subject were realigned using the first scan as a reference. The six parameters of this rigid body transformation were estimated using a least squares approach (Friston *et al*., 1995*a*). This approach is based on an approximate linear relationship between the images and their partial derivatives with respect to parameters of the transformation. Following realignment all images were transformed into a standard space (Talairach and Tournoux, 1988). The spatial normalization involves linear and non-linear three-dimensional transformations to match each scan to a reference image that already conforms to the standard space (Friston *et al*., 1995*a*). Each image was smoothed to account for the variation in normal gyral anatomy using a Gaussian filter (FWHM 16 mm for all directions). In the stereotaxic standard space, each voxel was $2 \times 2 \times 4$ mm in size.

After the appropriate design matrix had been specified, the condition effects were estimated according to the general linear model at each and every voxel (Friston *et al*., 1995*b*). Differences in global cerebral blood flow between scans were removed by analysis of covariance with global flow as a confounding variable (Friston *et al*., 1990). A systematic difference among subjects was also removed as a confounding effect. After the confounding effects had been removed, adjusted rCBF images were subjected to the following analysis.

Eigenimage analysis

To characterize the general pattern of the variance matrix across different conditions without any assumption, principal components analysis (eigenimage analysis) was applied to the adjusted rCBF images averaged across subjects (Friston *et al*., 1993). Each principal component can be described in a spatial domain (eigenimage) or a profile over conditions (condition loading). From this analysis we looked for the most predominant changes introduced by the experimental design.

Subtraction analysis

To test the hypothesis about the specific regional effects of the condition, the estimates were compared using linear contrast. The resulting set of voxel values for each contrast constitutes a statistical parametric map of the t statistic, and these were then transformed to the unit normal distribution (Z score) and thresholded at 3.09. The significance of each region was estimated using the probability that the peak height observed could have occurred by chance and/or that the observed number of contiguous voxels could have occurred by chance over the entire volume analysed (Friston *et al*., 1994). A corrected P value of 0.05 was used as the final threshold for significance. Activated areas below this level were considered to represent trends.

To identify the cortical areas activated with each movement task, three kinds of linear contrasts were examined. First, all movement conditions were individually compared with the rest condition, and four linear contrasts were done: 1–0, 2–0, 3–0 and 4–0, where 0 stands for the rest condition and the other numbers stand for all movement tasks shown in Table 1. Secondly, to identify the areas selectively activated by sequential movements, each sequential condition was contrasted with simple movement and three more contrasts were done: 2–1, 3–1, 4–1. Finally, the more complex sequences were contrasted with the simple sequence and two more contrasts were done: 4–2, 3–2.

Characterization of different patterns

We employed multiple regression analysis to identify the regions functionally related in different patterns. Adjusted rCBF values at the local maximal point in two representative regions were used as separate covariates. We chose the regions most strongly correlated with the first and second eigenimages as representative covariates (i.e. the contralateral sensorimotor cortex from the first eigenimage and the ipsilateral precuneus from the second eigenimage). Because the purpose of this analysis was to determine the functional correlation between different cortical areas, no correction for multiple comparisons was adopted in this analysis. Therefore, this analysis is useful in order to characterize descriptively the activated regions into different patterns of activity across conditions.

To determine the different relationships with complexity for the activated areas found in the multiple regression analysis, we chose the contrast having the largest difference of condition loading score (i.e. sequence-12 compared with rest) to identify cortical regions. Then searches for local

maximal foci in each activated area followed, and their coordinates were plotted. These local maximal foci are effectively regions of interest. Because the FWHM was 16 mm for all directions, the value in a single pixel represents the value in a circular region of interest ~16 mm in diameter.

Results

The subjects did not make any errors in the performance of the simple repetitive movement and sequence-4. The mean percentage of correct taps was >98% in all movement conditions (Table 2).

Table 2 *Performance of sequential finger movements*

Task	Errors (%)
Simple	0
Sequence-4	0
Sequence-12	0.50 ± 1.3
Sequence-16	1.78 ± 2.8

Values are mean ± SD for percentage of errors (% = number of errors/total taps×100).

Eigenimage analysis

Figure 1A shows the first principal component in the spatial domain (i.e. first eigenimage). The distribution of the eigenvalues shows that the first component can explain 80.2% of the total variance–covariance structure (Fig. 1B). This eigenimage included the bilateral SM1, premotor cortex, SMA, posterior and medial parietal cortex, contralateral basal ganglia (putamen) and ipsilateral cerebellum. The condition loading scores associated with the first eigenimage were characterized by a monotonic change with increasing sequence length, but showed saturation after sequence-12 (Fig. 1C). The second eigenvalue accounted for 10.7% of the total variance–covariance structure. This eigenimage included the bilateral precuneus, the parietal and premotor cortices and the cerebellum (Fig. 1D). The condition loading scores associated with this second eigenimage showed an

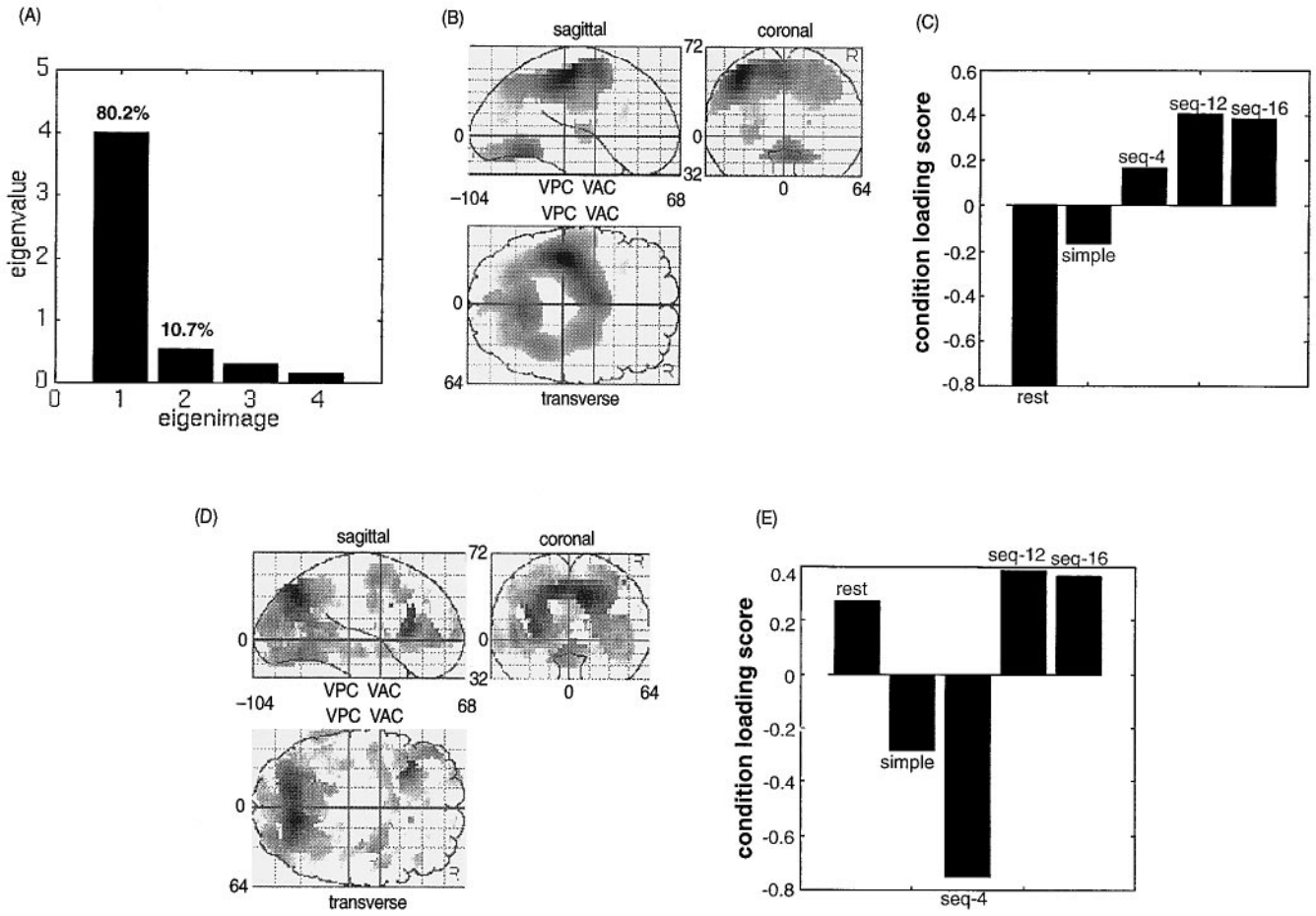

Fig. 1. Principal component analysis. (**A**) Distribution of the eigenvalues showing the first eigenimage (80.2% of the total variance-covariance), and the second eigenimage (10.7%). (**B**) Positive component of the first principal component (eigenimage). (**C**) Component score across conditions of the first eigenimage showing a monotonic increase with saturation after sequence-12. (**D**) Positive component of the second principal component (eigenimage). (**E**) Component score across conditions of the second eigenimage showing the main change between sequence-4 and sequence-12 and between sequence-4 and sequence-16.

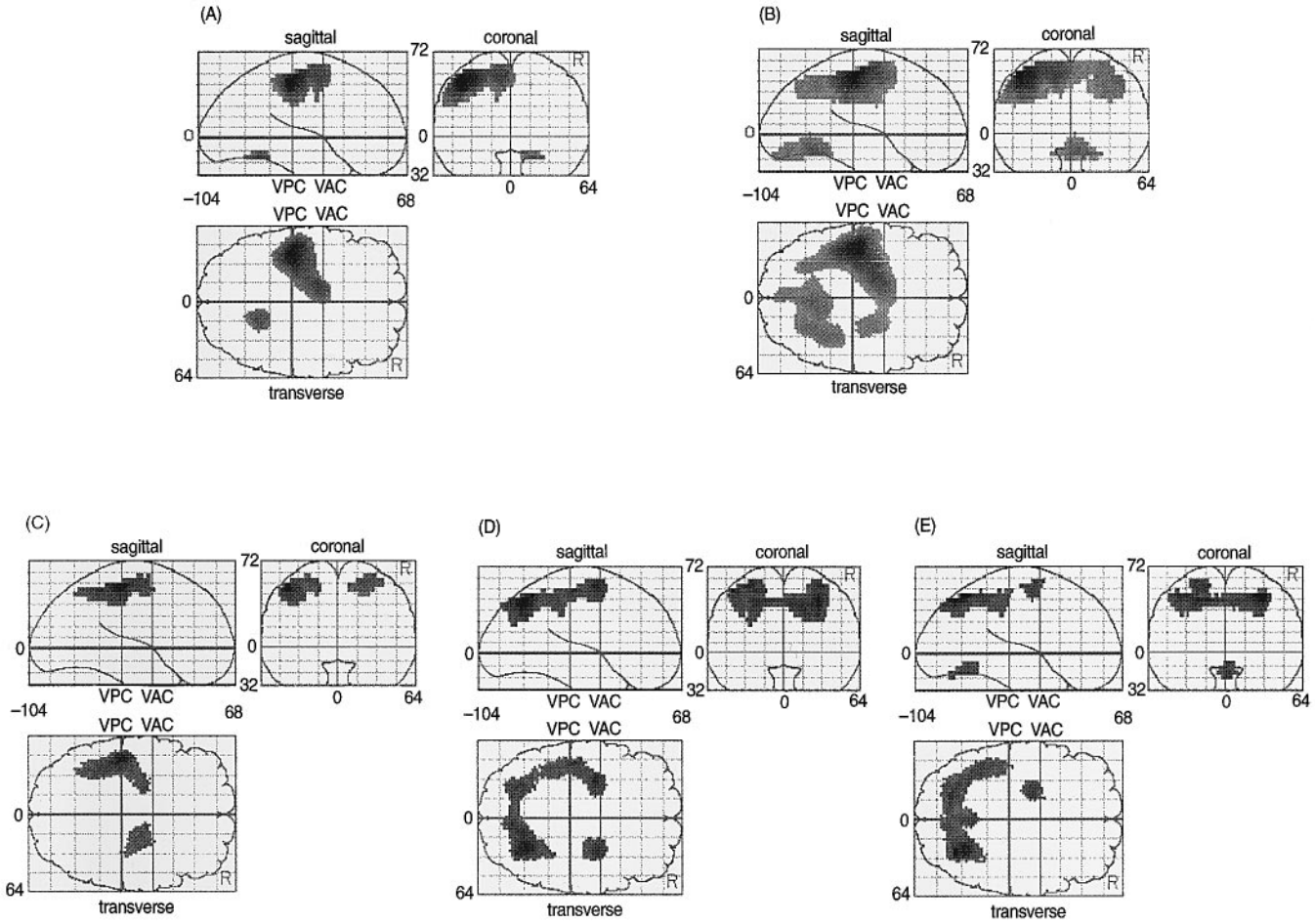

Fig. 2 Statistical parametric maps (SPMs) for increasing rCBF in the subtraction analysis showing the significantly activated areas. (**A**) Areas with increase in rCBF during simple repetitive movement compared with rest condition. (**B**) Areas with increase in rCBF during sequence-12 compared with rest condition. (**C**) Areas with increase in rCBF during sequence-4 compared with simple movement. (**D**) Areas with increase in rCBF during sequence-12 compared with simple movement. (**E**) Areas with increase in rCBF during sequence-16 compared with simple movement. The voxels displayed have *Z*-values exceeding the significance threshold of 3.09 with a Bonferroni correction for multiple comparisons ($P = 0.05$). The SPMs are displayed in the anatomical space of Talairach and Tournoux (1988) as a maximum intensity projection viewed from the right side (sagittal view), the back (coronal view) and the top (transverse view) of the brain. VAC = vertical line passing through the anterior commissure; VPC = vertical line passing through the posterior commissure.

abrupt change between sequence-4 and more complex sequences. Although the second eigenvalue was smaller than unity, the network included in its eigenimage was related more to sequential conditions.

Subtraction analysis

Increases in rCBF (activation) in simple movement, compared with rest, were observed in the contralateral SM1, dorsal premotor cortex, posterior SMA and ipsilateral cerebellar cortex (Fig. 2A). In addition to these areas, the subtraction of each sequential task compared with the rest condition showed increases in rCBF in the bilateral parietal cortex, ipsilateral dorsal premotor cortex, contralateral anterior SMA, ipsilateral SMA, precuneus and cerebellar vermis. Figure 2B shows the most representative contrast, a 12-length sequence compared with rest, based on the result of eigenimage analysis, because saturation occurred between 12 and 16 length sequences.

To find any significant differences between 'simple' and 'sequential' conditions we did a subtraction analysis of each sequential condition with simple repetitive movement. The bilateral motor and premotor cortex were significantly activated in sequence-4. As the length of the sequence increased, the ipsilateral premotor cortex, bilateral posterior and superior parietal cortex, and cerebellar vermis showed a progressive increase of activation. Although the ipsilateral premotor cortex was not activated during sequence-16, there was activation just below the threshold (Fig. 2C–E; Table 4).

The subtraction analysis between sequential conditions did not show any significant activation at threshold $Z = 3.09$ when corrected for the number of comparisons. Without such correction, the linear contrast between sequence-16 and sequence-12 compared with sequence-4 showed activation in

the cerebellum and precuneus. Below this threshold, the posterior parietal and premotor cortex were also identified. All these areas were also included in the second eigenimage.

Characterization of different patterns

To identify the regions that were functionally related to different patterns, we used multiple regression analysis with two covariates as a representative region for each pattern: contralateral sensorimotor cortex from the first eigenimage, and ipsilateral precuneus from the second eigenimage. The first covariate showed an increase in rCBF involving the bilateral SM1, contralateral premotor and posterior SMA, basal ganglia and cerebellum. The second covariate showed an increase in rCBF in the posterior parietal and precuneus bilaterally and ipsilateral premotor cortex. Selected regions identified in this analysis area are shown in Fig. 3.

Because the main pattern in the principal components analysis showed the highest component score in sequence-12, this condition was chosen to identify the areas to be plotted (Tables 3 and 4). For all areas identified by subtraction analysis, the adjusted rCBF was plotted in the comparison of sequence-12 with rest. All areas included in the first pattern had two steps of increase in rCBF: (i) from rest to simple movement, and (ii) from simple movement to sequence-4; in addition, the contralateral premotor cortex and cerebellum showed an rCBF increase across sequential conditions (from sequence-4 to sequence-12). Because all these areas showed the main changes between simple movement and sequence-4 they are most probably related to execution of movement, and they can be identified as 'executive areas' (Fig. 3A). Areas included in the second pattern did not have any increase in rCBF from rest to simple movement, but the main rCBF change was from sequence-4 to sequence-12, both conditions using the same number of fingers. The activation of these areas was functionally related to performance of sequences, so they can be identified as 'sequence-selective areas' (Fig. 3B). The concordant results from characterization of the plots and multiple regression analysis support the view that two different patterns exist.

Discussion

Task performance

Sequential motor tasks require motor learning, and their execution is characterized by a preprogrammed process that takes advantage of that learning (Halsband *et al*., 1993). Movement complexity might be understood in a variety of ways. As an index of complexity in our study, we were interested in sequence length, so we varied the length of the sequences while controlling the rate, rhythm and total number of movements. In this study the subjects practised the tasks before PET scanning to avoid any learning effect during the scan. All sequences were prelearned to the same level. Because all movement conditions were paced at a relatively slow speed (0.5 Hz), attention was required to perform the sequences correctly. The need for attention argues against automaticity of the motor performance. Subject performance was very good, as demonstrated by the small percentage of errors (Table 2). Even though errors were infrequent, their increase with increased length of the sequence argues that task complexity increases with sequence length.

Distinct sets of activated areas

Changes in rCBF have been correlated with presumed neuronal activity in different studies (Fox and Raichle, 1984; Price *et al*., 1992; Ibañez *et al*., 1995; Sadato *et al*., 1996*b*). These studies assumed a direct relationship between the rate of a stimulus and the neuronal electrical response, although this may not hold in certain ranges of frequencies, and showed that rCBF changes can be used as an indirect index of brain work. The CBF response of the non-primary cortices to the frequency of the stimulus may differ from that of the primary sensory cortices. During the presentation of heard words, the rCBF response in an area of the left posterior superior temporal gyrus (Wernicke's area) was primarily dependent on the occurrence of words regardless of their rate of presentation, whereas the primary auditory cortices showed a linear increase in rCBF response as the rate of presentation of heard words increased (Price *et al*., 1992). The authors concluded that time-dependent sensory signals detected in the primary auditory cortices were transformed into a time-invariant output that was channelled to a functionally specialized region (Wernicke's area). In the motor system, Sadato *et al*. (1996*b*) found a rapid increase in rCBF in the SM1 and cerebellum during auditory-cued, repetitive opponent finger tapping at a frequency of up to 2 Hz, with saturation of the rCBF at higher frequencies. On the other hand, the rCBF changes were largest in the SMA, anterior cingulate gyrus and right prefrontal regions during very slow movements (0.25 and 0.5 Hz) and declined at higher frequencies. The authors speculated that this was the result of a change in the character of the movement from reactive to predictive.

In the present study, by changing one parameter (i.e. length of the sequence) we tried to differentiate the cortical areas involved in motor execution of simple and sequential movements and cortical areas related to longer sequential tasks, which presumably correspond to different functions. Two different patterns of activation were identified with multiple regression analysis, and each different pattern could explain different aspects of sequence performance.

Eigenimage

Eigenimages can be interpreted as a predominant distributed neural network, which is introduced by the experimental design. They can give useful information about general tendency or change in both temporal and spatial domains, but it is impossible to extract localized changes specific to

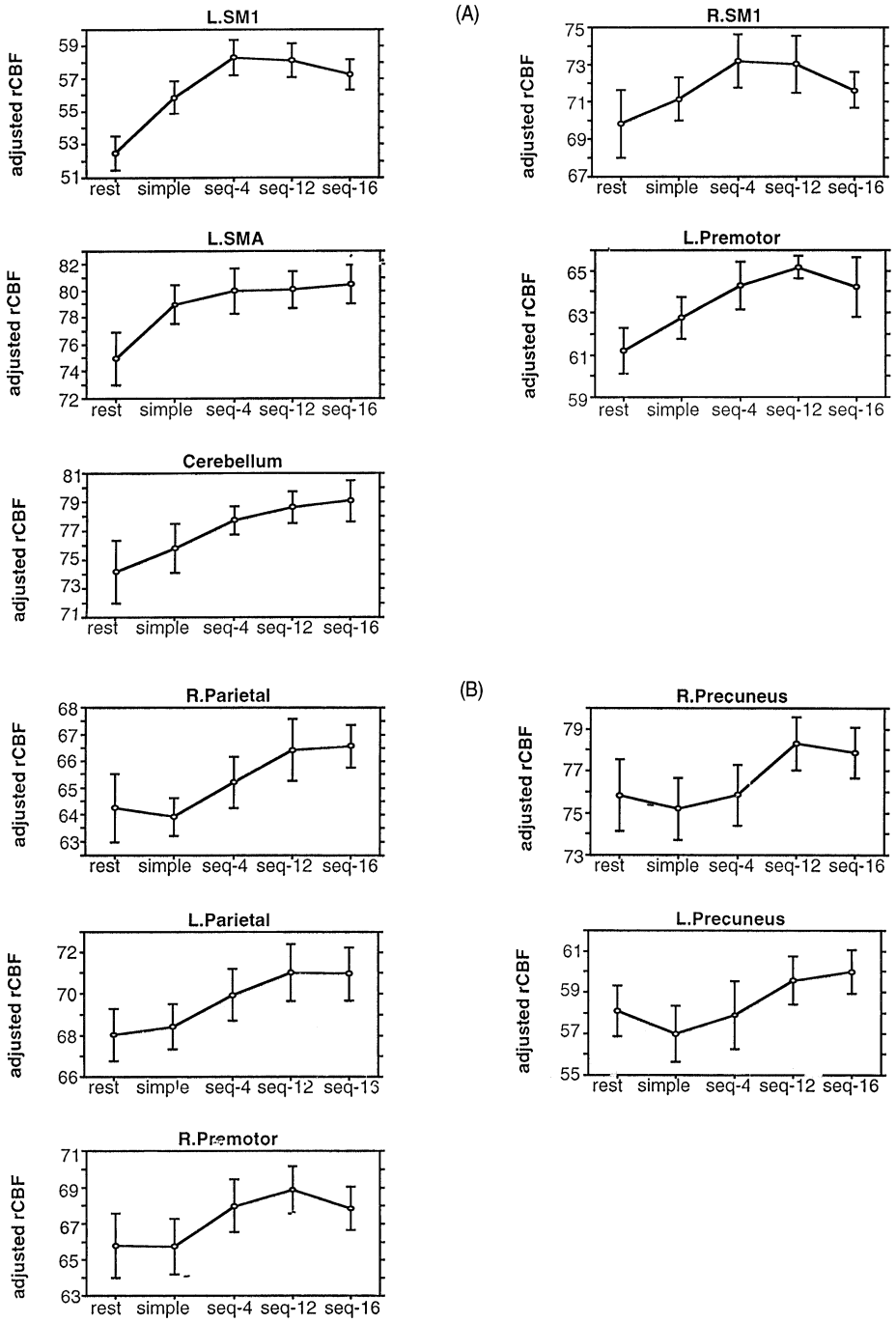

Fig. 3 The mean adjusted rCBF and standard deviations in cortical regions from subtraction of sequence-12 versus rest condition, showing two different patterns of activation across conditions. Values are plotted at the voxel of maximum *Z*-score in each region. (**A**) Executive areas. (**B**) Sequence-selective areas.

different motor tasks from this analysis. The first eigenimage can be interpreted as the most predominant change across conditions relating to the execution of motor sequences, and it includes most of the areas involved in motor and sequence performance. It was characterized by a monotonic increase of activation across conditions, but showed saturation after sequence-12. These findings may suggest that after a task of particular length or complexity no additional activation is necessary to perform sequential movements, or that rCBF has reached its maximum and cannot increase further. A different interpretation of the condition loading score might be explained by one change between rest and movement and another change between simple and sequential movement simply due to the use of more fingers. However, the loading score also changed from sequence-4 to sequence-12 and from sequence-4 to sequence-16, both sets of sequences using the same number of fingers.

The second eigenimage can be interpreted as activation of

Table 3 *Activation of different brain regions by sequential finger movements: comparison of sequence-12 versus rest condition*

Location	Talairach coordinates*			Adjusted mean rCBF (ml/min/100 ml)					Z-score*	% change
	x,	*y*,	*z*	Rest	Simple	Seq-4	Seq-12	Seq-16		
SM1 L	–44	–24	48	63.19	67.52	70.18	69.80	69.09	8.033	9.72
PMd L	–16	–8	52	71.12	74.39	76.20	76.23	76.57	6.598	6.82
pSMA L	–10	–8	56	74.96	78.97	80.00	80.09	80.47	5.591	6.50
Cerebellum R	6	–60	–12	78.77	80.85	82.36	83.41	83.68	5.331	5.67
Parietal L†	–26	–64	44	69.53	70.19	71.72	72.73	72.68	4.588	4.49
Parietal R†	26	–64	40	72.81	73.04	74.48	75.89	75.38	4.753	4.14
PMd R	22	–8	56	70.63	71.01	73.43	73.75	73.01	4.123	4.32
SM1 R	24	–18	48	69.81	71.19	73.21	73.04	71.62	4.298	4.50
SMA R	4	–2	52	83.49	85.77	88.03	87.50	87.78	4.382	4.64

SM1, primary sensorimotor cortex; SMA, supplementary motor area; pSMA, posterior supplementary motor area; PMd, dorsal premotor cortex; L, left; R, right. *Talairach coordinates and *Z* score of peak activation. †Brodmann area 7 according to the atlas of Talairach and Tournoux (1988).

Table 4 *Activation of different brain regions by sequential finger movements: comparison of sequence-12 versus simple movement*

Location	Talairach coordinates*			Adjusted mean rCBF (ml/min/100 ml)					Z score*	% change
	x,	*y*,	*z*	Rest	Simple	Seq-4	Seq-12	Seq-16		
SM1 L	–44	–22	52	52.47	55.85	58.31	58.13	57.25	4.471	4.04
Parietal L†	–26	–66	44	68.03	68.41	69.94	71.03	70.97	4.215	3.75
Parietal R†	28	–66	44	64.24	63.92	65.21	66.42	66.56	4.881	3.84
PMd L	–32	–6	56	61.19	62.76	64.29	65.18	64.22	4.465	3.81
PMd R	28	–8	56	65.79	65.74	67.99	68.88	67.86	4.278	4.66
Precuneus L	–18	–76	44	58.09	56.99	57.90	59.58	59.99	4.009	4.42
Precuneus R	14	–68	40	75.83	75.18	75.85	78.31	77.88	4.352	4.09
Vermis ‡	2	–60	–16	74.16	75.81	77.74	78.66	79.09	4.233	4.26

SM1, primary sensorimotor cortex; PMd, dorsal premotor cortex; L, left; R, right. *Talairach coordinates and *Z* score of peak activation. †Brodmann area 7 according to the atlas of Talairach and Tournoux (1988). ‡Coordinates of local maximal points, *Z*-scores and percentage change in rCBF were calculated for the comparison of sequence-16 versus simple movement.

areas related to sequence length, because the main change in the condition loading score was between sequence-4 and sequence-12 and between sequence-4 and sequence-16. Activation of most of the areas in the second eigenimage is related to performance of sequential movement (i.e. bilateral posterior parietal, precuneus and premotor cortex). The contralateral prefrontal cortex appears in this eigenimage. Its plot shows involvement only with sequential movements, as for the other areas in this eigenimage. Prefrontal cortex activation is involved in the selection of movement or preparation to move, and is related to activation of the posterior parietal cortex with sustained attention (Frith *et al*., 1991; Stephan *et al*., 1995; Coull *et al*., 1996).

Executive areas

The bilateral SM1 and posterior SMA showed an increase in rCBF from rest to simple movement, and another increase from simple movement to sequence-4. The contralateral premotor cortex and cerebellum showed the same pattern of increase in rCBF, although they also had an additional increase in rCBF across sequential tasks (Fig. 3A). This kind of increase in rCBF, with the main changes being between simple movement using two fingers and sequential conditions using five fingers, suggests that it is caused by execution of sequential movements of the fingers.

The primary motor and sensory cortex play an important role in both simple and complex movement (Shibasaki *et al*., 1993; Kawashima and Fukuda, 1994; Remy *et al*., 1994). Electrophysiological studies (Kitamura *et al*., 1993) have shown increased amplitude of the negative slow activity preceding complex movement compared with simple movement at both the midline vertex and the precentral areas bilaterally, possibly corresponding to the SMA and SM1, respectively. The results suggest that both SMA and SM1 play an active role in the preparation of the complex sequential movement. Although this hypothesis was only partly supported by the PET study (Shibasaki *et al*., 1993), it is consistent with our results, where SM1 showed higher activation during sequential tasks than during simple movement. Another possible explanation of this result might be the different number of fingers involved in each condition.

Simple movement in the present study consisted of tapping index finger to thumb, so only two fingers were involved. In contrast, sequential conditions involved all fingers of the right hand in their execution, and only the length of the unit sequence increased as an index of complexity.

A recent study using repetitive TMS over different human motor areas while subjects performed repetitive finger movements and complex finger sequences indicated that not only the SMA but also the primary motor cortex (contralateral M1) is more involved in the processing of complex sequential finger movements than in simple repetitive finger movements (Gerloff *et al*., 1997). These results are consistent with the different involvement of M1 with different degrees of complexity and are compatible with our results showing a greater increase in rCBF with sequential movements than with simple repetitive movements. Although there are reports from TMS and EEG studies showing more involvement of the contralateral sensorimotor cortex with sequences of increasing complexity (Corwell *et al*., 1996; Manganotti *et al*., 1997), we did not find a significant increase in rCBF in the contralateral motor cortex with performance of longer sequences. This may indicate that the results from EEG and TMS studies were at least in part due to premotor activation.

Activation of the ipsilateral SM1 during execution of sequential movements in the present study confirms the findings of previous reports (Kim *et al*., 1993; Shibasaki *et al*., 1993; Sadato *et al*., 1996*a*). Activation of the ipsilateral SM1 is more anterior than activation of the contralateral SM1, probably because sensory feedback is absent on the ipsilateral side (Sadato *et al*., 1996*a*). Another possibility is that the ipsilateral representation of the hand is truly anterior to the representation of the contralateral hand. A study using TMS indicates that this may be true (Wassermann *et al*., 1994). In a recent study using TMS while subjects performed a simple and a complex sequence of finger movements, Chen *et al*. (1997) found that ipsilateral M1 stimulation caused more errors in the complex than in the simple sequence. Although these results demonstrate the involvement of ipsilateral M1 in the performance of motor sequences, the different disruption of each sequence may be explained, at least in part, by premotor activation.

Different studies suggest a specially important role of the SMA in the planning and/or execution of complex voluntary movements in the human (Orgogozo and Larsen, 1979; Roland *et al*., 1980*b*; Deiber *et al*., 1991; Grafton *et al*., 1992). The SMA can be segregated into at least two functionally different parts, the pre-SMA (anterior) and SMA proper (posterior). They are roughly divided by the vertical anterior commissure line (Deiber *et al*., 1991). The posterior SMA is more 'executive' and it is activated during repetitive movements (Colebatch *et al*., 1991; Matelli *et al*., 1993). Tanji and Shima (1994) provided strong evidence for the involvement of the posterior SMA in the programming of sequential movements. Another report (Grafton *et al*., 1992) supports the involvement of the posterior SMA in the complexity of voluntary movements.

In the present study, the SMA showed greater activation for sequential tasks (sequence-4) than for simple movement, but it did not show any change across longer sequences. Although the peak activation was located in the posterior SMA (Table 3), the anterior SMA could be also involved in the performance of sequential movements (Fig. 2B). The SMA might take part in the preparation of internally referenced or remembered motor acts. This finding is in agreement with the results of previous reports (Shibasaki *et al*., 1993; Sadato *et al*., 1996*a*). Shibasaki *et al*. (1993) found significantly more activation in the posterior SMA during sequential finger movements compared with simultaneous finger movements. Sadato *et al*. (1996*a*) found consistently posterior SMA activation regardless of the sequence length, but their study design did not include simple movements. The correlation between SMA activity and sequential movements can be explained by either preparation or programming. After the overlearning of motor sequences, internal preparation is needed to perform the sequential movements from memory or internal representation. We conclude that activation of the posterior SMA is related to the execution of the sequence, regardless of its length, and simple repetitive movement might require different preparation and motor programming than sequential tasks.

The contralateral premotor cortex and cerebellum showed the same pattern of activation, but showed some increase in rCBF across sequential tasks (Fig. 3A). Previous studies support the role of the PMC in different motor tasks (Kawashima *et al*., 1994; Stephan *et al*., 1995). An interesting finding in our study is the increasing activation of the PMC with longer sequences. There is clinical evidence for a critical role of the PMC in the temporal organization of sequential movements (Halsband and Freund, 1990; Halsband *et al*., 1993), with left hemisphere dominance for the temporal aspects of motor programming. Moreover, previous PET studies (Grafton *et al*., 1992; Shibasaki *et al*., 1993) support a role of the PMC in the generation of motor sequences from memory that fit into a precise plan. These results suggest that performance of longer sequences, because they are more difficult, requires more motor programming in sequence organization.

Different PET studies have demonstrated an increase in rCBF in the anterior cerebellar hemisphere with voluntary movements of the fingers (Fox *et al*., 1985; Seitz *et al*., 1990; Grafton *et al*., 1992; Shibasaki *et al*., 1993). Some other studies (Pardo *et al*., 1991; Roland, 1993) have shown cerebellar activation related to non-motor tasks requiring working memory. Sadato *et al*. (1996*a*) found that a linear increase in rCBF in the cerebellar vermis was related to the increasing length of a motor sequential task, and they suggested a non-executive meaning of this activation. Our result is similar, indicating a role both in execution and in sequence control. For voluntary limb movements, the cerebellum is clearly important for the temporal order of and precision in the execution of motor programmes.

Sequence-selective areas

Activation of the ipsilateral premotor, bilateral posterior parietal cortex and precuneus was related to the performance of sequences, showing no change from rest during the performance of simple movement (Fig. 3B). These findings suggest that these areas are involved in sequence processing rather than execution of movement. Sequential finger movements are more complex than simple repetitive movements in that the forthcoming sequence has to be programmed from memorised information (Mushiake *et al*., 1991) and then executed.

The premotor cortex is considered to be the centre of complex skilled movements, and it can be separated into discrete areas (Dum and Strick, 1991), although most previous PET studies have not differentiated them. The dorsal premotor cortex in the primate is located more caudodorsally, and the ventral premotor cortex is located more in the postarcuate region. Each premotor area is a nodal point for a discrete set of afferent inputs from subcortical nuclei and from cortical areas comprising different systems of movement control (Dum and Strick, 1991; He *et al*., 1993). The dorsal premotor cortex of the monkey exhibits prominent motor set-related activity (Kurata and Wise, 1988; Mushiake *et al*., 1991). Sequence-specific neurons were also more numerous in the dorsal premotor cortex and SMA, although neuronal activity during a visually triggered task was more prominent in the ventral premotor cortex (Dum and Strick, 1991; Mushiake *et al*., 1991). SMA neurons were more active when the sequence was remembered and self-determined. It has been suggested that there is some functional specialization of the SMA for controlling internally referenced motor output and of the PMC for the control of externally referenced motor acts. In our study activation was primarily in the dorsal premotor cortex, which is compatible with the primate studies.

Sadato *et al*. (1996*a*) reported that dorsal PMC activation progressively increased on the side ipsilateral to the movement as the length of the unit sequence increased. The right PMC is activated during functioning of visuospatial working memory (Jonides *et al*., 1993) and bilaterally, more prominently on the right, during learning and performing overlearned sequences (Jenkins *et al*., 1994). These results suggest a role for the right PMC in storing motor sequences in a working memory buffer.

Imagining and executing movements activate the intermediate and caudal parts of the superior parietal lobe (Brodmann area 7, including the precuneus) (Stephan *et al*., 1995). There are several lines of evidence that Brodmann area 7 is involved in multimodal integration of external information and that it provides a sensory representation of extrapersonal space. PET studies have shown that Brodmann area 7 is related to motor selection with auditory cues as well as with visual cues, based on integration of spatial information (Deiber *et al*., 1991; Grafton *et al*., 1992), and the dorsal parietal cortex and precuneus respond to an increment of the spatial complexity of the task (Grafton *et al*., 1992). Stephan *et al*. (1995) found activation in the superior parietal lobe that was thought to be associated with visuomotor integration or with shifts in the direction of attention in space. Because subjects had their eyes closed during the tasks, any form of visuomotor integration during motor tasks or of shifts of attention in space would depend on memorized spatial information.

Another issue relating to this parietal area is attention. Jenkins *et al*. (1994) found activation of Brodmann area 7 bilaterally in auditory-cued, complex sequential finger tapping, and this activation was more prominent during learning of the new sequence. They speculated that this activation might be related to spatial attention to the fingers, because subjects had to pay more attention to their fingers during learning than during the well-learned task. Although parietal activation could be related to learning itself, their results, as in our study, were more consistent with spatial attention to the movement selection in a sequential task. Parietal areas might play a role in the temporal aspects of the sequence and contribute to the integration of sensory information into the movement sequence, to ensure that each movement occurs after successful completion of the preceding one. This is consistent with recent findings that patients with parietal cortex damage have difficulty predicting the time required to perform differentiated finger movements (Sirigu *et al*., 1996).

In the present study, longer sequences might need more attention, as increased complexity implies an increase in the number of possible choices, and there is more difficulty in selecting the correct finger for each movement within the given sequence. Posterior superior and medial parietal areas could be recruited in storing information about the motor sequence (Sadato *et al*., 1996*a*). Since there is a strong inter-regional relationship between the parietal and premotor cortices, Brodmann area 7 may have a role in selecting and monitoring a sequence with on-line reference to a working memory in the right PMC.

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