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Attention and stimulus characteristics determine the locus of motor-sequence encoding
A PET study

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Summary
PET revealed the effects of stimulus characteristics on the neural substrate of motor learning. Right-handed subjects performed a serial reaction time task with colour-coded stimuli to eliminate the potential for learned eye-movements. The task was performed with the right hand under two different conditions. In one condition, subjects simultaneously performed a distractor task. Although they did show behavioural evidence of learning, they were not explicitly aware of the stimulus–response sequence. In the second condition, there was no distractor task, and seven out of the 11 subjects then became explicitly aware of the stimulus sequence. Metabolic correlates of learning were distinct in the two conditions. When learning was implicit under dual-task conditions, learning-related changes were observed in left motor and supplementary motor cortex as well as in the putamen. These regions are similar to those observed in a previous study in which the stimuli were cued by spatial position. Under single-task conditions, metabolic changes were found in the right prefrontal cortex and premotor cortex, as well as in the temporal lobe. A similar shift to the right hemisphere was observed in the spatial study during single-task learning. However, explicit learning of the task with colour stimuli activated more ventral regions. The areas supporting motor-sequence learning are contingent on both stimulus properties and attentional constraints.

Keywords: motor learning; sequencing; PET; human; explicit learning; implicit learning

Abbreviations: BG = basal ganglia; SMA = supplementary motor area; SRT = serial reaction time

Introduction
A prominent tenet of learning research over the past decade has been the hypothesis that there are multiple systems for learning and memory. A basic dichotomy has been proposed to distinguish between explicit and implicit forms of learning. Explicit memory refers to those memories in which the subject has explicit access to the learning experience and is aware of previous encounters with a particular set of stimuli. In contrast, implicit memory refers to those memories in which the subject need not be aware of changes in performance that come about through prior experience.

It has been argued in a number of cognitive domains that explicit and implicit memories involve dissociable memory systems. Numerous studies have shown that patients with lesions in medial temporal and diencephalic structures are able to acquire various skills despite dramatic memory impairment on explicit tests of recall (*see* Squire, 1986; Schacter and Tulving, 1994). In one commonly employed experimental paradigm, patients show normal learning on skilled tasks such as mirror-reading or mirror-drawing. However, when given explicit memory tests for materials presented during the learning sessions, such as a recognition test for the words presented during mirror-reading, the patients exhibit marked deficits (e.g. Cohen and Squire, 1980).

Nissen *et al*. (1987) introduced a motor-sequencing task, the serial reaction-time (SRT) task, to compare implicit and explicit learning. The SRT task has proved quite useful in both the study of cognitive operations involved in sequence learning and efforts to identify the neural mechanisms associated with motor learning. In the basic form of this task, subjects view a computer monitor, on which a stimulus

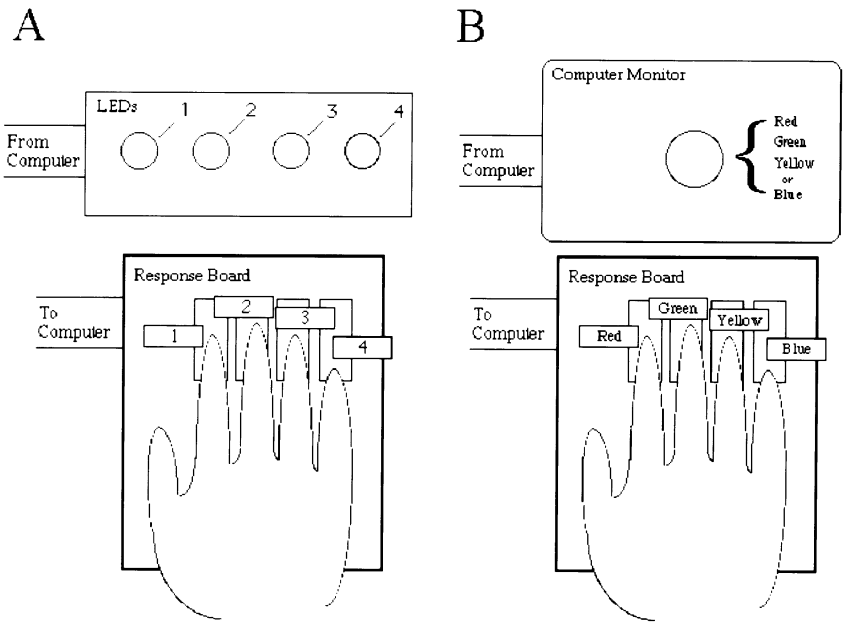

Fig. 1 Experimental layout of the SRT task. In the spatial version of the task (**A**), four spatially-separated light-emitting diodes (LEDs) are used to indicate one of the four required key presses. In the colour version of the task (**B**), any one of the four different colours is presented at a single fixation point. Each colour cues a different finger movement. For both versions of the task the stimulus-response pattern is learned prior to initiation of PET imaging and presentation of sequentially organized stimuli. Thus, learning effects are related to stimulus order rather than the mapping of stimuli to motor effectors.

appears at one of four positions. The four fingers of the right hand are placed on a four-key response board, and the subjects are instructed to press the key which corresponds to the spatial position of the stimulus. For example, if the stimulus appears at the leftmost position, the subject would respond by making a keypress with the index finger. The stimuli can either appear at randomly selected positions or, in separate blocks, they can follow a predetermined sequence that cycles repeatedly. A schematic diagram of the typical experimental setup is shown in Fig. 1A. For the present study, subjects were asked to respond according to stimulus colour rather than stimulus position; this removed the spatial component of the stimuli. This version of the experiment is depicted in Fig. 1B. In a typical SRT experiment, subjects complete a set of blocks with sequenced patterns ('sequence blocks') and then a set of blocks in which the stimulus locations are allocated randomly ('random blocks'). A performance measure of learning is obtained by comparing mean response latencies in the final sequence blocks with the latencies in the immediately subsequent random blocks. Response latencies invariably become slower upon this transition.

In addition to this performance measure, subjects are queried as to their explicit knowledge of the sequence. As would be expected, the decrease in response times during the sequence blocks is much larger when subjects have developed explicit knowledge; indeed, in some experiments, reaction times become minimal as subjects anticipate the next stimulus location. In addition, numerous experiments have shown that learning, as manifest by faster reaction times during sequence blocks in comparison with random blocks, is also evident even in the absence of any explicit knowledge of the sequence (Willingham *et al.*, 1989; Cohen *et al.*, 1990; Curran and Keele, 1993).

Awareness during the SRT task has been prevented in several different ways. One method is to use a long sequence (e.g. cycle every 12 elements), to exceed the capabilities of working memory (Nissen and Bullemer, 1987). A second method has been based on neuropsychological manipulations; patients with Korsakoff's disease (Nissen and Bullemer, 1987) as well as normal subjects who have been administered the amnesic agent, scopolamine (Nissen *et al.*, 1987), show implicit learning without having any awareness of the sequence.

A third method involves the use of a secondary, distractor task such as tone counting. With this task, a tone is presented between each motor response and the next stimulus and the subject is asked to keep an internal count of the tones which match a target frequency. This secondary task is quite demanding and has proved to be extremely effective in preventing awareness (e.g. Nissen and Bullemer, 1987; Cohen *et al.*, 1990). Thus, the SRT task provides a common behavioural paradigm in which motor-sequence learning can occur either explicitly or implicitly.

Curran and Keele (1993) investigated the relationship between these two forms of learning. Two groups of subjets were compared using the SRT task. One group was explicitly told in advance that there would be a sequence on some

blocks and was even shown the six-element sequence. The other group was not given this information. A *post hoc* division was made in the second group, separating those who reported some awareness from those who reported little, or no, awareness.

Without attentional distraction, the informed subjects showed much faster learning of the sequence, as evidenced by the fast drop in the mean response latency and the large cost when the random condition was re-introduced. Most important, when they were subsequently tested under conditions with attentional distraction, no advantage was observed for the informed or aware subjects. From these results, Curran and Keele (1993) proposed that independent learning systems were involved under the two conditions. The declarative knowledge used by the informed subjects when the distractor task was not present did not help them when they needed to attend to the tone-counting task.

In a previous study (Grafton *et al.*, 1995), we used PET to determine which neural systems are associated with performance changes as people learn novel motor sequences in the SRT task. All subjects completed one set of blocks under dual-task conditions and then a second set under single-task conditions. Within each set of 17 blocks, the stimuli were randomly chosen for the first seven blocks, the next eight blocks used the sequenced pattern, and the final two blocks used random stimuli. Different sequences were used for the dual- and single-task conditions.

In these SRT experiments, learning can be assessed by changes in reaction time over the sequence blocks and, more importantly, by increases in latency following the final shift from a sequence block to a random one. Both measures of learning were substantially larger during single-task performance, reflecting the fact that, in this condition, many subjects became aware of the sequence and were able to anticipate the forthcoming stimulus. Nonetheless, learning was also evident in the dual-task blocks as shown by the significant increase in reaction time following the shift from sequence to random blocks. Learning was entirely implicit here; no subjects became aware of the sequential nature of the stimuli.

PET scans were obtained during every third block. We focused on the three scans obtained during the sequence blocks for the dual-task condition and the three scans obtained during the sequence blocks for the single-task condition. In particular, what metabolic changes were correlated with the performance changes? We did not use a subtractive procedure. Rather, we looked for linear increases in metabolic activity that occurred as response latency decreased during the blocks in which the stimuli followed a fixed sequence. Areas that showed a similar linear trend during the random blocks were ruled out as reflecting changes not specific to sequence learning.

As anticipated by many of the behavioural studies, increases in activation occurred in distinct neural systems for the two conditions. Under dual-task conditions, when awareness was blocked, learning-related increases in rCBF were located in contralateral motor-effector areas including the motor cortex, supplementary motor area and putamen, consistent with the hypothesis that non-declarative motor learning occurs in cerebral areas that control limb movements. In the single-task condition, learning-related increases in rCBF were present in the right dorsolateral prefrontal cortex, right premotor cortex, right ventral putamen and biparieto-occipital cortex. The right dorsolateral prefrontal and parietal areas have been previously implicated in spatial working memory. Seven out of 12 subjects developed awareness of the sequence during this condition.

These findings were the starting point for the current study. In particular, we were interested in two related issues. Do the neural systems involved in sequence learning depend on the stimulus characteristics used to define the sequence? If so, are these stimulus-specific effects similar, when learning takes place under attentional distraction, to those when attention is directed solely to the sequencing task?

As in the previous study, we compared metabolic correlates of sequence learning under dual- and single-task conditions. The critical manipulation for assessing the contribution of the stimulus properties was to substitute colours for each of the spatial positions used in the previous study. Thus, whereas the leftmost position had required a keypress with the index finger in the spatial version of the SRT task, this same response was now associated with the colour red. Similarly, the other three positions were replaced by three distinct colours. In this way, the responses were essentially identical to what we had required in the spatial task, but the stimuli were now colours, all presented at a central location on the computer monitor.

It is important to bear in mind that while the colour version of the SRT task removes the spatial component of the stimuli, the responses retain their spatial component. One question surrounding the SRT literature is whether the sequence learning involves learning a series of perceptual or motor events (e.g. Willingham *et al.*, 1989; Keele *et al.*, 1995). Based on a set of transfer studies, Willingham *et al.* (1989) argued that learning can not be restricted to either level, but rather arises as subjects learn a series of stimulus–response contingencies. By changing the stimulus characteristics, but not the responses, we should gain further insight on this issue. If sequence learning is primarily perceptual in nature, we would expect to find the change in stimulus properties to lie outside areas associated with motor control; if sequence learning is primarily motoric in nature, however, then the changes should be restricted to motor areas. The results of Willingham *et al.* (1989) would suggest differences in both perceptual and motor areas.

Further motivation for the current study is inspired by considering potential component processes involved in sequence learning. These putative components, at least without attentional distraction, are processes that are attributed to working memory. Recent work reported in the human and animal literature suggests that the neural correlates of working memory may vary as a function of

stimulus characteristics. In a series of PET studies, Jonides, Smith and colleagues have reported a laterality effect for spatial and object working memory (summarized in Smith *et al*., 1995). In both versions of their experiment, subjects performed a variant of a matching-to-sample task in which they judged whether a test item matched one of two previously presented samples. For the spatial task, the comparisons were made on the basis of stimulus location; for the object task, the comparisons were made on the basis of shape. The dorsolateral prefrontal cortex was significantly activated in both tasks, a result consistent with the hypothesis that these tasks involve working memory. Interestingly, this prefrontal activation was in the right hemisphere during the spatial task and in the left hemisphere during the object task.

On the other hand, in single-cell studies with primates, Goldman-Rakic and her colleagues (*see* Wilson *et al.*, 1993) have argued for a dorsal/ventral distinction in prefrontal cortex between spatial and object working memory. Cells in the dorsal lateral prefrontal cortex continue to respond during a delay period in which the animal has to remember the location of a recently seen stimulus. Cells in the more ventral inferior convexity continue to respond during the delay period when the animal must remember the shape of the recently seen stimulus. Their work did not address issues related to laterality.

In our previous study with spatial stimuli (Grafton *et al*., 1995), we found dramatic laterality effects for implicit and explicit learning. When learning was implicit, increases in activation occur mainly in the left hemisphere, which controls the right hand with which subjects made their responses. However when learning was explicit, activation shifted to the right hemisphere. This is consistent with the ideas of Smith *et al*. (1995) and Goldman-Rakic and colleagues (in Wilson *et al*., 1993): the task required learning about the sequential nature of spatial events and the foci were in areas of the human brain that are somewhat homologous to the regions identified in primate studies as essential for spatial working memory. Moreover, activation in dorsolateral prefrontal cortex in the right hemisphere has been observed in a number of studies involving recognition (Tulving *et al*., 1994; Kapur *et al*., 1995), a process that could be expected to be involved during explicit sequence learning.

The current study provides a novel test of the hypothesis that neural loci of working memory are task-dependent. While keeping the responses exactly the same, we can assess whether the metabolic correlates of sequence learning shift when the successive responses are cued by colours rather than positions. According to the Jonides hypothesis, we would expect to see a shift from right prefrontal regions to left prefrontal regions. According to the Goldman-Rakic hypothesis, we should expect to see a shift from dorsal to ventral regions within prefrontal cortex. Since these two hypotheses are not mutually exclusive, it remains possible that both could be supported.

Material and methods

Healthy subjects performed the SRT task during PET scanning under both dual- and single-task conditions. Eleven normal right-handed subjects (seven men and four women; mean age $\pm$SD, 21.5$\pm$3.2 years) participated after informed consent was obtained in accordance with the USC Institutional Review Board, which approved the study. Subjects underwent a neurological history and physical examination to rule out any pre-existing conditions, and completed a handedness questionnaire prior to the study (Oldfield, 1971).

SRT task

The procedure was identical to that of our previous study with the exception of the presentation of the SRT stimuli (Grafton *et al.*, 1995). Each subject was positioned in the scanner and a computer monitor was then mounted above their chest. A series of coloured circles subtending ~1° of visual angle appeared, in turn, at the centre of the screen. Each circle was filled with one of four colours (red, green, yellow or blue) which indicated the index, middle, ring and little finger, respectively, as shown in Fig. 1B. Subjects were taught to press the appropriate response key as rapidly as possible.

All of the subjects used their right dominant hand. The right arm was extended parallel to the body axis and rested on a table with the index, middle, ring and little finger resting on four response keys. A fixed inter-stimulus interval of 1500 ms was used so that the number of movements per block (and PET scan) was held constant. Circles were always removed 1000 ms after appearing. By using an invariant inter-stimulus interval and stimulus exposure duration, the sensory input as well as the number of responses produced per unit time was held constant.

A block of trials involved 84 responses and the sequence of stimulus colours was either presented randomly or in a six-element pattern that repeated continuously. The overall structure of the sequence was identical to that in the spatial study, in that two colours were presented twice within each six-element cycle and two were presented once. An example is red–green–red–yellow–green–blue. Previous work has shown that this type of sequence, composed of both ambiguous and unique pairwise associations, can be learned under conditions of distraction without awareness (Cohen *et al.*, 1990). Within the sequence blocks, this six-element pattern would be repeated 14 times, with the starting position randomized across blocks. There were four different sequences with two assigned to each subject, one for the dual-task blocks and one for the single-task blocks. On random blocks the stimulus locations were chosen randomly with the constraint that no location be chosen twice in succession.

For each of the dual- and single-task conditions subjects first performed seven blocks of random trials, then eight blocks of sequence trials and finally two blocks of random trials. Behavioural indices of learning were derived by

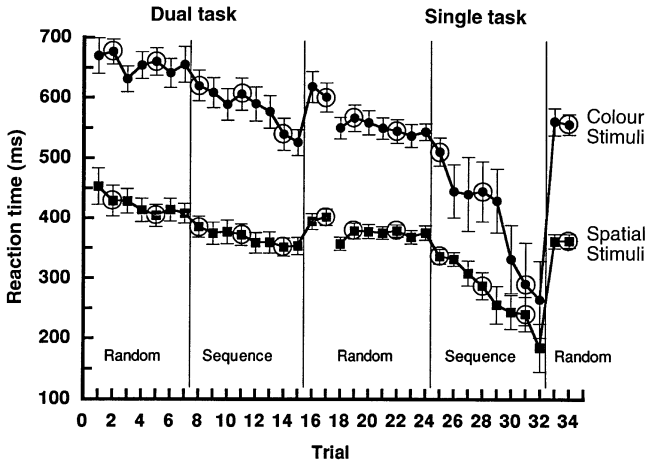

Fig. 2 Performance assessment of SRT task. Mean±SD block is plotted for each SRT. The upper curves summarize results for colour stimuli, the lower curves for spatial stimuli (also presented in Grafton *et al*., 1995). The left half of the Fig. summarizes data from the dual task (tone counting) and the right half is from the distraction-free task (single task). For both, the blocks begin with sequences presented in random order (seven blocks), then sequential order (eight blocks) and finally random order (two blocks). Blocks during PET imaging are circled. Reaction times for colour stimuli are greater than those for spatial stimuli, secondary to overall greater task difficulty in the former. For both stimuli, under single and dual conditions, there are significant reductions of reaction times with presentation of an ordered sequence. The increase of reaction times with subsequent random trials confirms that the changes are learning effects.

comparing median reaction times on sequence blocks with those obtained on random blocks.

In the dual-task condition, the subjects were required, concurrently, to monitor a stream of 50 ms audible tones, and to keep track of the number of low pitched tones. Targets for the secondary task were 200 Hz pure tones. Distractors were 1000 Hz pure tones. The presentation of the visual and auditory stimuli were made asynchronous by varying the delay between the onset of a coloured circle and the onset of the tones. Intervals of 1100, 1200 or 1300 ms separated the two events. Between 50% and 75% of the tones were targets in the dual task. The number of target tones was randomly varied between blocks, eliminating the possibility of a learning effect related to the secondary task. Prior to these imaging experiments, a significant learning effect without development of awareness was confirmed for this modified SRT dual task (using a fixed inter-stimulus interval) in a group of eight subjects tested outside the scanner environment.

In the single-task condition, the motor-sequence task was performed alone and subjects were instructed to ignore the tones. Tones were also presented in the same manner as in the dual-task blocks, but only at the distractor frequency. Inclusion of the tones in the single-task blocks was to approximate the auditory stimulation while providing minimal attentional interference. Different sequences were used in the single and dual tasks and subjects were always unaware that a sequence would be presented prior to the start of each condition. Since subjects consistently did not become aware of the sequence in the dual-task condition, they were first tested on this condition. Subjects were interviewed at the end of the experiment to determine whether they had become aware of the sequence, either in the dual- or single-task phases. In addition, they were asked to generate the sequence on the response board.

Imaging

Images of regional cerebral blood flow (rCBF) were obtained by bolus intravenous injections of 35 mCi radioactive water ($H_2{}^{15}O$) using a modified autoradiographical method and a Siemens 953/A scanner with a measured in-plane resolution of 7.5 mm and a between-plane resolution of 5 mm after reconstruction (Herscovitch *et al.*, 1983; Raichle *et al.*, 1983). Scans were performed at a 15° angle relative to the anterior–posterior commissural line. The field of view extended from the vertex to the mid-cerebellum. However, because of the steep angle, the inferior/orbital frontal and posterior parietal cortex were not in view.

Twelve sequential PET scans (six in the dual task, then six in the single task) of 90 s duration were obtained every 10 min. For each scan, tracer injection, imaging and a block of trials were started simultaneously. Two additional blocks of trials were presented in the 10 min interval between sequential PET scans. The relationship between block type and scanning is summarized in Fig. 2. Blood samples were not obtained. Images of radioactive counts were used to estimate relative changes in rCBF, as described previously (Fox *et al.*, 1984; Mazziotta *et al.*, 1985). Attenuation was corrected-for, using boundary information from the sinogram of each scan.

Image analysis

Data processing required three steps: (i) within-subject coregistration of images to remove interscan movement errors; (ii) between-subject image coregistration to pool data and provide a common reference space for describing the location of responses; (iii) statistical analysis to identify learning-related changes in rCBF. Images from each individual were aligned (within-subject) using an automated registration algorithm as previously described (Woods *et al.*, 1992). The 12 coregistered images from each subject were averaged to generate a mean rCBF image for each individual. These mean images were then coregistered onto a reference PET atlas centred and scaled to match the Talairach atlas (Talairach and Tournoux, 1988). This between-subject coregistration uses an ‘affine’ fitting algorithm incorporating 12 parameters (three rotations, three translations and three scalars along axes specified by an additional three parameters) (Woods *et al.*, 1993). The transformation matrices were then applied to all of the original rCBF scans to match them to the target Talairach atlas. Images were then smoothed to a

final image resolution of 20 mm full width at half maximum, and were normalized (within-subject) to a common global value. After smoothing with a Gaussian filter, there were ~120 grey-matter resolving elements as defined previously (Worsley *et al.*, 1992).

A repeated-measures analysis of variance (ANOVA) was chosen *a priori* as an appropriate statistical model for identifying learning-related changes in rCBF. The experimental design was set-up so that learning would occur during the third, fourth and fifth PET scan of each condition (dual- and single-task conditions; *see* Fig. 1). As the performance measure demonstrated a longitudinal improvement in reaction time during these three scans, we used a model that would identify longitudinal changes in rCBF over the same trials and scans. In other words, we consider changes in brain activity to be indicative of learning when there are concurrent changes in performance. Longitudinal changes in rCBF, during the three scans when learning was occurring, were determined on a pixel-by-pixel basis with a multivariate repeated-measures (ANOVA) (Maxwell and Delaney, 1990). For the dual-task learning occurred during Scans 3, 4 and 5 and for the single task it occurred during Scans 9, 10 and 11. A full multivariate ANOVA was calculated rather than a mixed model F test to avoid errors related to assumptions of compound symmetry that can occur when variance is pooled. An omnibus F test for changes in rCBF activity during the three sequence scans was first calculated and a significance threshold of $P < 0.005$ was selected. Once a pixel location was identified as showing a significant change during the three time points, the next step was to define the nature of the changes in rCBF.

With only three time points, the number of possible linear contrasts that could best describe the significant differences noted on the omnibus test were limited to two models. A site of significant rCBF change could be described in terms of a monotonic model (Scan 3 – Scan 1 = 0) or by a quadratic model (2 × Scan 2 – Scan 1–Scan 3 = 0). Of these, the quadratic model has no measurable performance correlate which shows a quadratic change, so it is not clear what this quadratic type of change represents. Therefore, we included only those areas in which the significant change in rCBF was best explained by a monotonic model, analogous to the changes observed in the performance data. To do this, the pixels with a significance above the omnibus statistical threshold, and where a linear contrast F test for a monotonic contrast was greater than a quadratic model F test were displayed in pseudocolour onto an anatomical reference atlas.

Monotonic increases and decreases in rCBF were both considered. The pixel with maximum significance within each site was identified and used to localize responses with respect to the Talairach coordinates. To investigate time effects unrelated to the learning of sequences, the three scans obtained during presentation of random trials were compared using the same omnibus F test. Sites with significant time effects unrelated to learning were excluded from the final analysis. These statistics were performed separately for the single- and dual-task conditions.

Categorical comparisons between subjects performing the SRT task with spatial versus colour stimuli were determined with unpaired t tests calculated on a pixel-by-pixel basis, without variance pooling or a Bonferroni correction. For the dual task, Scan 2 from the spatial and colour studies were compared, for the single task, Scan 8 was compared. These scans were chosen because they are obtained during random blocks when subjects were familiar with the task but had not had training with the sequence. Thus, the interpretation of differences between the two is not contaminated by the effects of colour- versus spatial-sequence learning. Instead, this analysis should identify sites specific to stimulus processing. A statistical threshold of $P < 0.005$ was used.

Statistical results were reconstructed in three dimensions with a single subject's MRI scan using the Advanced Visualization Software (Waltham, Mass., USA). Responses were digitally projected onto the MRI surface for improved visualization with respect to gyral anatomy.

Results

Performance

The average reaction times for each block are shown in Fig. 2. Blocks 1–17 represent performances during dual-task conditions, when subjects were counting low-pitched tones during the SRT task. Blocks 18–34 represent performances under single-task conditions, when tones were present but not counted. Note that the reaction times for the spatial version of the experiment are considerably shorter than those for the colour version, reflecting the greater stimulus response compatibility in the former experiment.

As Fig. 2 shows, the reaction time decreased across Blocks 8–15, the blocks in which the sequence was present. Though the decrease was significant [$t(10) = 4.87$; $P < 0.01$], this measure of learning is problematic because it may reflect familiarization with aspects of the task and generalized practice effects, having little to do with the acquisition of the sequence. For this reason, the change in reaction time between the final sequence blocks and the subsequent random blocks is used to assess specific sequence learning. Averages of the final two sequence blocks and the final two random blocks (blocks 14 and 15, and 16 and 17) were computed and compared. Switching from sequence blocks to random blocks caused the mean reaction time to increase by 92 ms, [$t(10) = 6.11$; $P < 0.01$]. Thus, we find reliable learning with the colour-coded sequences, and this learning is implicit by definition, since none of the subjects reported any awareness of the sequence during the dual-task phase of the experiment. It is important to reiterate that the decrease in response latencies over the initial 15 blocks can not be solely attributed to learning the correspondence between particular colours and finger movements. If this were so, we would not have expected the increase for the final random blocks.

The same statistical procedure was applied to the data from the single-task condition. Again there was a significant decrease in the reaction time over the sequence blocks [block 24 versus block 32: t (10) = 4.16; $P < 0.01$]. More importantly, the sequence–random transition was also significant [t (10) = 3.69; $P < 0.01$]. As shown in Fig. 2, learning in the single-task condition is more dramatic. Indeed, some subjects had mean reaction times <50 ms indicating that they were anticipating the identity of the stimulus, and the sequence–random transitions were numerically larger. The mean increase over the 11 subjects was 296 ms. However, the learning scores from the single-task condition are likely to come from a bimodal distribution. In the debriefing session, seven of the 11 subjects reported becoming aware of the sequence during the single-task condition and, indeed, were able to reproduce at least part of the sequence. The other four subjects had no awareness of the sequence. The increase in reaction time during the random block was 448 ms for the seven aware subjects and 31 ms for the four unaware subjects. None of the subjects reported any awareness of the sequence during the dual-task condition.

To test the assumption that learning is related to the sequences in the dual-task condition, statistical analyses were performed on each of the six elements. Several aspects of the data support the hypothesis that subjects are learning the specific sequences implicitly, rather than overall probabilities or unique associations within the sequence. First, responses to all six elements of the sequence show significant improvement during the sequential blocks. Had subjects simply learned that some key presses were more likely than others, decreases in reaction would be expected only on more frequent keys. Furthermore, there was no significant effect of element on reaction time improvement during the sequence blocks ($F = 0.58$; $P > 0.5$). In fact, on average the reaction-time improvements for unique transitions (i.e. 3→2 or 4→1 in the sequence 1→2→1→3→2→4) were slightly but non-significantly smaller than for the other non-unique transitions.

Dual task imaging

First, the areas that showed significant monotonic changes in blood flow across the three scans obtained during sequence blocks (Scans 3, 4 and 5), but not across the random blocks (Scans 1, 2 and 6) were identified. In the dual-task condition, learning-related increases in rCBF were observed primarily in both frontal and parietal areas of the left hemisphere, as shown in Fig. 3 and detailed in Table 1.

Three of the sites show a striking correspondence to the learning-related areas identified in our previous PET study using spatial stimuli (Grafton *et al.*, 1995). In both experiments, increases in rCBF were observed in the supplementary motor area (SMA), motor cortex and subcortical putamen/thalamus, suggesting these motor areas represent implicit sequential motor actions that are independent of the stimuli used to cue movements. The Talairach locations were not identical for the two experiments. These small differences in spatial location may be methodological, related to insufficient resolution provided by the PET procedure, or possibly due to actual differences in the location of sequence representations with different stimuli.

Additional increases of rCBF were located in bilateral inferior parietal cortex. During learning, decreases in rCBF were found in the cerebellum, bilateral middle temporal cortex and inferior occipital areas. Similar decreases in bilateral middle temporal cortex had also been observed in the spatial study.

Single task imaging

When the sequence was learned without the secondary task, allowing subjects to attend fully to the stimuli, a very different set of regions showed increasing activation across the sequence blocks (Scans 9, 10 and 11). Under single-task conditions, learning-related increases in rCBF were prominent in right hemisphere, namely in the premotor, inferior frontal, anterior cingulate, inferior temporal and occipital regions, as shown in Fig. 4. The only left-sided increase was in the anterior cingulate cortex.

These areas, listed in Table 2, differ substantially from the sites observed in our previous experiment using spatial stimuli (Grafton *et al.*, 1995). In the prior study, parietal/occipital and dorsolateral prefrontal cortex showed learning-related changes, consistent with the notion that explicit sequence representations of spatially cued movements are represented in areas dedicated to spatial working memory. In the current study, a more ventral set of areas, including inferior occipital/temporal and inferior frontal areas, emerges. This is consistent with a model of working memory in which non-spatial information is represented in a ventral stream routed through the inferior temporal cortex and the inferior prefrontal cortex. In both cases, areas with increased rCBF during explicit learning are located in the right hemisphere.

Because the behavioural data suggested that there were two populations of subjects in the single-task condition, those who became aware of the sequence and those who remained unaware, separate analyses were performed on these two groups. In both groups, all of the areas listed in Table 2 showed a significant linear trend in activation, except for the left anterior cingulate, the left dorsal occipital cortex and right thalamus. For these three areas, the linear trend in the subgroup analysis was only significant for those subjects who developed awareness of the sequence. Limiting the analysis to just those subjects who became aware did not reveal any additional areas other than those listed in Table 2. Given that the unaware group contained only four subjects and the analysis was therefore limited in power, there is little indication that the results are an amalgamation of separate sets of processes. Awareness, itself, appears to play little role in the recruitment of neural systems. However, it must be kept in mind that, with the current design, we are unable to determine the point at which the seven subjects developed awareness. The dip in response times after the second

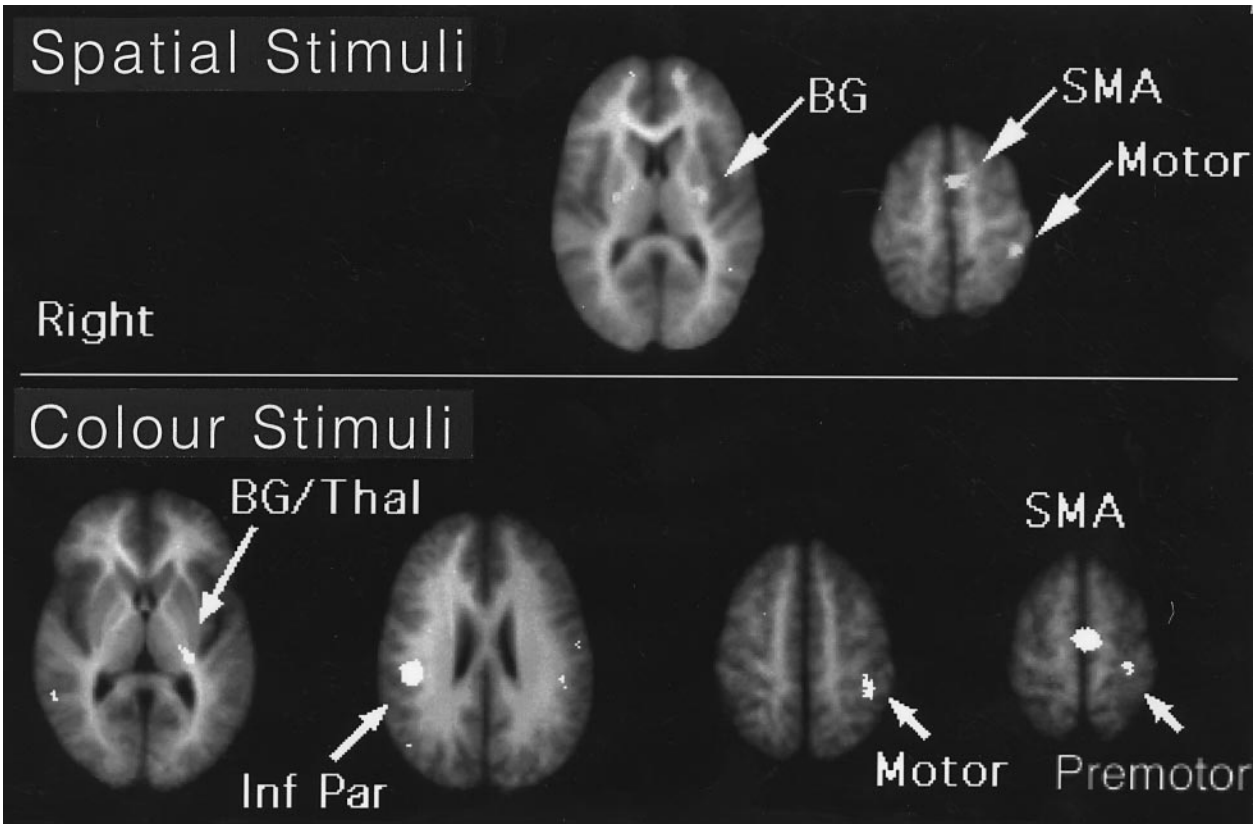

Fig. 3 Sequence learning with attentional interference. Significant rCBF increases ($P < 0.005$) are superimposed on an MRI reference atlas centred in Talairach coordinates. For both spatial and colour stimuli, there are significant increases in activity in the precentral gyrus (motor), supplementary motor area (SMA) and basal ganglia (BG) as subjects learn the sequential order of the stimuli. This learning is implicit, as subjects never become aware of a sequential order in the stimuli. Irrespective of stimulus features, common changes occur primarily in motor effector areas. The location of the SMA increase in rCBF during the spatial task is more rostral than for the colour stimuli. For colour stimuli there is an additional increase in rCBF in the right inferior parietal cortex (Inf Par). Upper images are 12 and 57 mm above the anterior–posterior commissural axis; lower images are 34, 3 and 60 mm above the anterior–posterior commissural axis. Upper row adapted from Grafton *et al*. (1995). Thal = thalamus.

sequence scan would suggest that awareness only developed near the end of training.

Stimulus characteristics

To determine the differences between using spatial and colour stimuli to designate discrete finger movements, subtractions across the spatial and colour studies were performed. The comparisons were based on the second scan within each condition, for the fifth random block, in both the dual and single task. Thus, no differences can be attributed to learning. Areas that are recruited in both the spatial and colour versions of the task would also not be detected by this comparison. Our experimental design does not achieve sufficient statistical power to demonstrate that population differences in learning spatial and colour cues is significant. However, the populations can be compared directly during the non-sequence blocks to determine the differences between using spatial and colour stimuli irrespective of learning.

The results are listed in Table 3. Finger movements in response to colour stimuli preferentially activated anterior cingulate (bilaterally), the left caudate, left premotor cortex, left inferior temporal cortex and left inferior parietal cortex amongst others. Comparable movements in response to spatial stimuli preferentially activated the left hippocampus, multiple occipital sites, right inferior frontal gyrus and right inferior parietal lobe. Since the finger movements in the two experiments were identical, these areas represent differences in stimulus processing or stimulus–response mapping. For almost all areas, the differences were significant whether a task was performed under single or dual conditions. The similarity between single- and dual-task lists suggests that attentional interference did not alter the systems used to process the stimuli and/or relate already learned arbitrary visual stimuli to discrete movements. Another important finding is that sites showing preferential activation for mapping colour stimuli to movements are not necessarily the same areas showing learning-related changes in rCBF.

Discussion

Two memory systems

We have confirmed that the neural systems associated with improved performance on a motor learning task depend on

Table 1 *Motor sequence learning with distraction of attention: colour stimuli*

Region (Brodmann area)	Talairach coordinates			rCBF (ml/min/100 g)			ANOVA (repeated measures)	
	x	*y*	*z*	Scan 3 (mean±SD)	Scan 4 (mean±SD)	Scan 5 (mean±SD)	*F*	*P*-value
Increasing rCBF								
L precentral gyrus (4/6)	−24	−31	60	54.40±3.16	55.10±3.38	56.23±2.91	23.24	0.0007
L SMA (6)*	−1	−16	57	63.93±3.09	65.28±3.89	65.79±3.46	29.29	0.0003
L sensorimotor (4)*	−31	−31	51	57.25±1.97	57.52±2.73	58.66±2.34	14.76	0.004
R inferior parietal (40)	48	−30	34	51.58±1.66	51.69±2.18	52.63±1.99	24.26	0.0006
L inferior parietal (40)	−43	−31	25	53.80±2.57	54.54±3.37	55.05±2.76	13.59	0.0042
L parietal operculum (40)	−51	−13	24	49.60±1.75	50.46±2.62	51.31±2.17	13.15	0.0046
R parietal operculum (40)	37	−28	19	52.87±2.22	53.64±1.81	54.95±2.04	23.73	0.0007
R parietal (40)	57	−18	15	45.56±1.57	46.31±2.90	46.99±1.91	21.63	0.001
L thalamus/putamen*	−21	−25	3	52.16±1.81	52.90±1.73	53.38±1.77	20.24	0.0015
Decreasing rCBF								
R middle occipital (19)	40	−75	18	43.94±1.85	43.08±2.18	42.80±1.79	21.42	0.001
L middle temporal (21)	−52	−57	13	47.79±1.96	47.10±1.93	46.40±1.87	17.4	0.002
R middle temporal (21)	49	−49	7	53.70±2.34	52.98±2.44	52.50±2.29	15.06	0.0031
L lingual (19)	−18	−51	−3	52.24±1.73	51.39±2.72	51.10±1.82	20.19	0.0012
L inferior temporal (20)	−54	−25	−15	51.40±2.49	50.24±3.24	49.28±1.95	22.8	0.0008
R fusiform (37)	24	−52	−18	61.76±3.09	60.45±2.61	60.62±3.31	22.49	0.0008
L cerebellar nuclei	−27	−52	−22	62.77±1.42	62.24±2.07	61.54±1.33	20.3	0.0011
R posterior cerebellum	46	−58	−25	45.96±3.73	44.94±3.80	44.27±3.46	33.34	0.0002

Brain areas demonstrating a significant longitudinal change of rCBF during sequence learning are reported in Talairach coordinates with corresponding Brodmann areas in parentheses (Talairach, 1988). Significance was determined with a repeated measures ANOVA across the three scans where the stimuli were presented in sequential order. None of these sites demonstrated significant changes of rCBF when stimuli were presented in random order. *Regions were significant in a previous study using spatial rather than colour stimuli (Grafton *et al*., 1995).

the availability of attentional processes. When such processes are available, explicit awareness of the sequence can occur along with the improvements in reaction time. When subjects are distracted by a tone-counting task, improvement in reaction times are still observed, but awareness of the sequence never occurs. Fig. 5 provides a schematic summary of the neural foci correlated with learning in the dual- and single-task conditions in both the current study and in Grafton *et al*. (1995).

As in our previous study, no common foci were seen in the dual- and single-task conditions. Learning-related changes in blood flow during learning with attentional distraction were primarily localized to motor regions in the contralateral hemisphere. Given the resolution of the SMA, foci cannot be unambiguously assigned to either hemisphere. In contrast, when the secondary task was eliminated, metabolic correlates of learning were observed in the ipsilateral hemisphere, localized to prefrontal cortex, premotor cortex and the temporal lobe. Together, the two studies provide strong evidence that dissociable neural systems are involved in implicit and explicit skill acquisition, an interpretation that resonates with a robust behavioural literature. In agreement with the results of Curran and Keele (1993), these neuroimaging data suggest that separate neural systems are involved in skill acquisition when sequence learning takes place under conditions of attentional distraction, as opposed to situations when the learning process is not disrupted by a secondary task.

The attentional components of the dual and single tasks differ considerably. In the former, the tones must be closely monitored while in the latter they should be ignored. One might argue that some of the differences between the dual and single tasks are associated with processes involved in ignoring the tones. However, it is unlikely that activation due to such inhibitory processes would show systematic changes restricted to the sequence blocks. On all single-task blocks, random and sequence, the subjects had to ignore the tones. Moreover, the neural systems involved in learning are identified by within-task comparisons, so attentional differences themselves are not responsible for the pattern of results. Because the pattern of activation was nearly identical for aware and unaware subjects in the single-task condition, it appears that the attentional components, and not awareness, determine which neural regions are correlated with sequence learning.

The loci for dual- and single-task learning, shown in Fig. 5, bear some correspondence to the 'What–Where' dichotomy proposed by Ungerleider and Mishkin (1982). During implicit learning, metabolic changes were observed in neighboring regions of motor cortex, SMA and putamen. Parietal lobe activation was also observed in the implicit conditions of both experiments, although the colour foci were considerably more ventral and bilateral. During single-task learning, there was significant activation in occipital, temporal, lateral premotor and prefrontal areas for both the spatial and colour sequences. One conjecture would be that

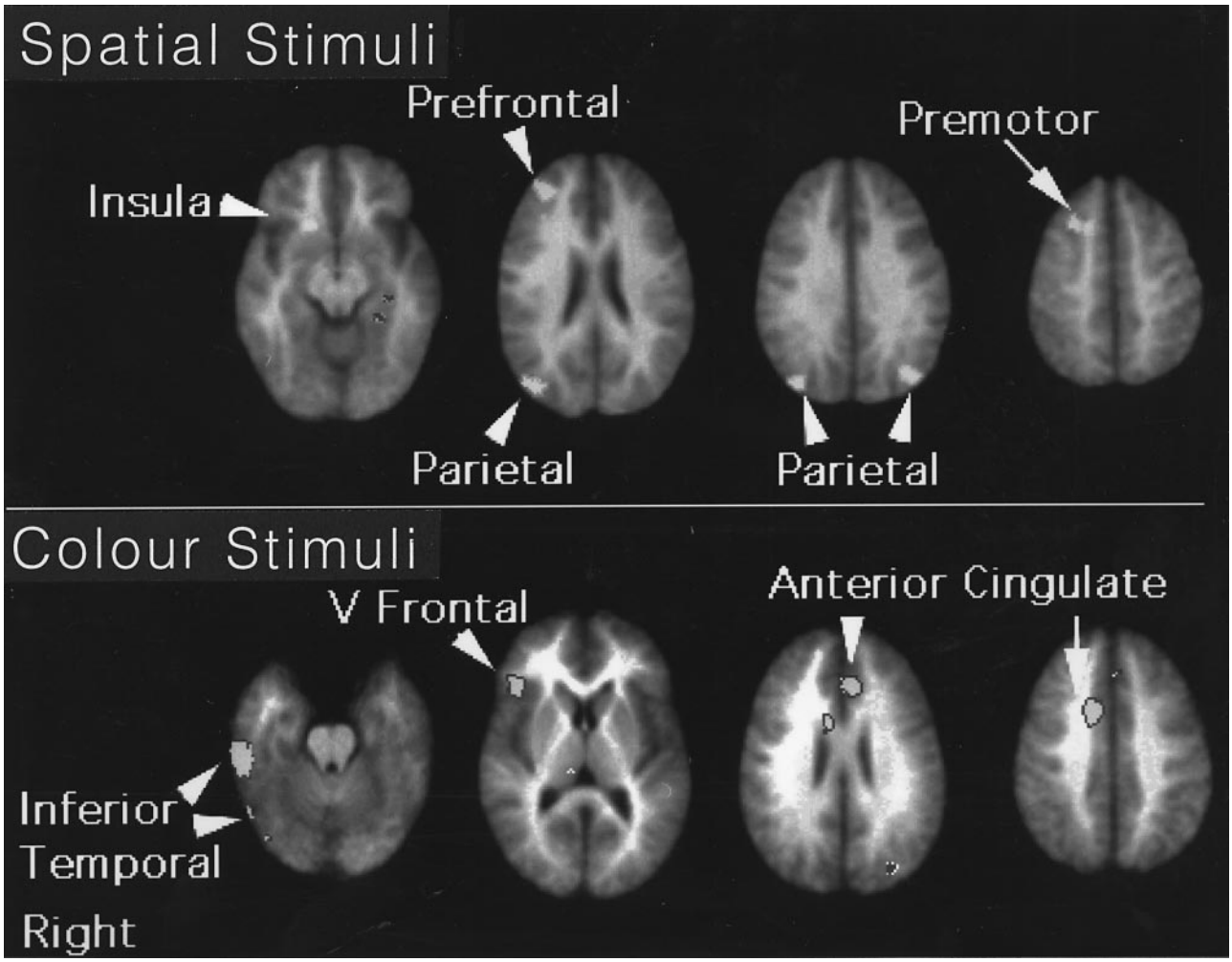

Fig. 4 Sequence learning without attentional interference. For spatial stimuli, rCBF increases during learning of ordered sequences are found in the parietal cortex (bilaterally), the right premotor cortex and right prefrontal cortex. These areas are implicated in spatial working memory. For colour stimuli rCBF increases are located in the right inferior temporal and frontal cortex as well as bilaterally in the anterior cingulate cortex. These areas are also active in other tasks of non-spatial working memory. Learning becomes explicit for approximately half of the subjects, who can verbally report the correct order of the stimuli. Upper images are –8, 26, 35 and 50 mm above the anterior–posterior commissural axis; the lower images are –18, 7, 19 and 34 mm above the anterior–posterior commissural axis. The upper row is adapted from Grafton *et al*. (1995).

the pathways underlying single- and dual-task learning reflect fundamentally different ways in which sequential behaviour is generated. Dual-task learning might be viewed as a form of spatial-motor priming in which movements to a series of successive locations are facilitated by preceding stimuli and responses. In contrast, when attention is not distracted, learning could be based on the identification of anticipated elements in the sequence.

An alternative conceptualization is that learning in these two types of conditions reflects different forms of association. The presence of the distractor tones may prevent the development of complex associations that are represented in the right hemisphere pathways. These associations could occur in the single-task condition as each colour is preceded by an invariant auditory stimulus. Note that, in accord with both of these hypotheses, the temporal lobe foci need not be interpreted as reflecting a specialized mechanism for motor-sequence learning. Rather, the temporal lobe may provide the long-term associations that can be exploited in the SRT task (Keele *et al*., 1996).

Alterations in the methodology insure that the behavioural changes are not simply reflecting learning a series of eye movements. With spatial sequences, subjects generally move their eyes from one location to another, thus learning is not necessarily restricted to the finger movements. By presenting the colours at a central location, the present experimental design eliminated eye movements. Nonetheless, we obtained behavioural evidence of learning and found many similarities in the general pattern of metabolic correlates of learning to those observed in our previous study (Grafton *et al.*, 1995). This extension to a non-spatial dimension further specifies what is learned. In accord with previous suggestions

Table 2 *Motor sequence learning without distraction of attention: colour stimuli*

Region (Brodmann area)	Talairach coordinates			rCBF (ml/min/100 g)			ANOVA (repeated measures)	
	x	*y*	*z*	Scan 9 (mean±SD)	Scan 10 (mean±SD)	Scan 11 (mean±SD)	*F*	*P*-value
Increasing rCBF								
R premotor (6)	18	12	60	44.43±1.86	44.50±2.55	45.29±2.20	13.85	0.004
R anterior cingulate (24/32)	10	6	34	47.85±2.72	49.36±2.51	49.67±2.53	53.91	0.00002
L anterior cingulate (24/32)	−4	28	19	52.45±3.80	54.03±3.98	54.81±3.36	30.29	0.0003
R inferior frontal (45)	40	19	7	62.13±3.62	62.94v4.04	64.22±4.53	20.51	0.002
R thalamus	3	−22	7	61.45±2.34	62.18±2.59	62.67±2.40	14.53	0.004
R inferior temporal (20)	55	−28	−18	44.52±3.60	44.95±3.20	46.70±3.04	53.22	0.00003
R inferior occipital (19/39)*	37	−78	−19	41.45±6.54	42.67±7.08	42.91±6.65	20.99	0.002
R inferior occipital (19)	49	−63	−21	34.67±2.85	35.22±3.15	35.58±3.13	21.73	0.0009
Decreasing rCBF								
R superior parietal (7)	13	−60	48	51.51±2.34	50.62±2.58	49.92±2.01	22.95	0.0008
L dorsal occipital (19)	−28	−78	24	52.98±2.74	51.97±2.18	51.89±2.25	24.29	0.0006
R posterior insula	25	−27	15	47.06±3.16	46.32±3.01	45.85±2.63	13.98	0.004
L anterior cerebellum	−9	−52	−12	58.36±3.89	57.78±3.18	56.67±3.14	17.5	0.002

Brain areas demonstrating a significant longitudinal change of rCBF during sequence learning are reported in Talairach coordinates with corresponding Brodmann areas in parentheses (Talairach, 1988). Significance was determined with a repeated measures ANOVA across the three scans where the stimuli were presented in sequential order. None of these sites demonstrated significant changes of rCBF when stimuli were presented in random order. *Region was significant in a previous study using spatial rather than colour stimuli (Grafton *et al*., 1995).

Table 3 *Differences of spatial and non-spatial stimuli to designate keyboard responses*

Region (Brodmann area)	Talairach coordinates			Dual task		Single task	
	x	*y*	*z*	*t*	*P*	*t*	*P*
Colour > spatial							
B anterior cingulate (24)	0	24	24	−3.53	0.00198	NS	
L inferior temporal gyrus (37)	−58	−39	−16	−4.51	0.00019	−3.23	0.0040
L frontal insula	−36	10	3	−3.28	0.0036	NS	
L caudate	−6	0	6	−3.29	0.00356	NS	
L middle frontal gyrus (10)	−22	49	24	−4.23	0.0004	−3.97	0.0007
L precentral gyrus (6)	−43	−3	27	−2.48	0.022	−3.79	0.0011
L inferior parietal lobule (40)	−42	−31	39	NS		−3.56	0.0020
R pulvinar thalamus	3	−33	10	−3.39	0.00279	−4.00	0.0008
R superior frontal gyrus (9)	22	45	30	−3.42	0.00261	−3.44	0.0029
Spatial > colour							
L hippocampus	−31	−25	−4	NS		3.36	0.0030
L middle occipital gyrus (19/39)	−46	−66	10	5.06	0.00009	2.94	0.0088
L superior occipital gyrus (19)	−33	−73	30	3.64	0.00173	4.23	0.0004
R occipital (17)	12	−85	6	5.65	0.00001	5.39	0.0001
R middle occipital gyrus (19/39)	43	−66	10	4.30	0.00066	4.49	0.0003
R inferior parietal lobule (40)	52	−22	27	4.74	0.00011	2.65	0.0160
R Inferior frontal gyrus (44/6)	39	6	28	3.35	0.00308	3.39	0.0030
R Superior occipital gyrus (19)	33	−75	30	3.93	0.00089	3.85	0.0013

Differences between using spatial and colour stimuli to designate specific finger movements. Locations are given in Talairach coordinates with the corresponding Brodmann areas in parentheses (Talairach, 1988). The differences between the spatial and colour stimuli during the dual task were obtained from the differences in Scan 2 between the two conditions; Scan 8 was used for the corresponding single-task comparisons. Significance determined by unpaired *t* test.

(Willingham *et al*., 1989; Keele *et al*., 1995), learning is not merely a series of stimulus locations. Rather, it occurs at a more abstract or response-related level.

One intriguing aspect of the PET findings is that the two systems appear to operate exclusively rather than in parallel; increases in rCBF in one set of areas do not seem to co-occur with increases in the other. Pascual-Leone *et al*. (1994) also found evidence that non-simultaneous systems were associated with implicit and explicit sequence learning. They mapped cortical regions with transcranial magnetic stimulation as subjects learned a 12-element sequence with the SRT. Subtle interviews were conducted after each block to determine when learning had become explicit, without leading the subjects to look for the sequences. The period of

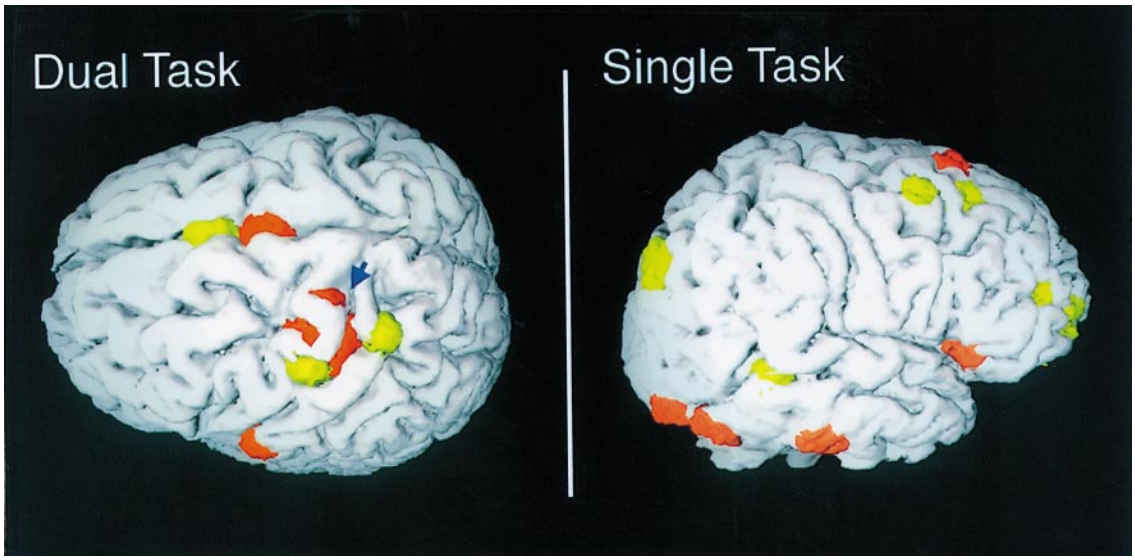

Fig. 5 Schematic three-dimensional reconstruction of learning-related increases in rCBF during SRT learning. Results of colour stimuli are shown in red and spatial stimuli are in yellow. Responses are enlarged and projected onto the surface of a single normal subject's MRI scan to aid visualization. The SRT dual-task learning with either type of stimulus leads to increases in rCBF in the motor cortex of the left hemisphere and left SMA (*left panel*). The blue arrow denotes the central sulcus. In addition, spatial stimuli recruit additional areas in the adjacent precentral premotor cortex, whereas colour stimuli recruit the adjacent postcentral sulcus (rostral parietal cortex). The SRT single-task learning (*right panel*) shows more dramatic changes in right hemisphere. Spatial learning changes are more distributed; they are located in the posterior temporal, dorsal prefrontal and posterior parietal cortex. Colour-sequence learning is associated with increases in rCBF in the inferior occipital and temporal cortex.

implicit learning was defined as the period before learning had become explicit but while the reaction time was decreasing. During implicit learning, the cortical output maps to the muscles producing the responses increased in extent. This did not happen in a control group, who performed the same task but without a sequence. However, once learning became explicit, the process of expansion reversed, and the maps returned to their baseline topography.

However, one caveat must be noted. While the experiments reported in Curran and Keele (1993) suggest independent systems, their behavioural indices of learning suggest that the two systems could operate in parallel. Similar findings have been reported by Schmidtke and Heuer (1996). The reason for this discrepancy between our PET results (exclusive systems) and these behavioural results (parallel systems) remains unclear, but may relate to the peculiarities of brain metabolism. Given that global blood flow is relatively constant, metabolic increases in one area may necessitate reduced activity in other regions. Thus, the large blood flow changes in the association cortex during learning without distraction would outweigh any additional changes in the motor effector areas.

Spatial and object pathways

In the dual-task condition, similar regions were associated with sequence learning in the spatial and colour experiments. Both experiments showed increased rCBF in the motor cortex, putamen, parietal and SMA. While foci within motor cortex and putamen showed considerable overlap, the differences in parietal cortex and SMA are intriguing. For the spatial experiment, the parietal focus is more dorsal, and the SMA region is more rostral. The colour experiment also revealed an additional increase in rCBF in dorsolateral premotor cortex, an area that is activated across a variety of 'movement versus rest' experiments (Roland *et al.*, 1980; Deiber *et al.*, 1991; Grafton *et al.*, 1996). These differences may reflect the effects of stimulus characteristics on implicit sequence learning. Recent neurophysiological and PET studies have suggested multiple motor subregions in these premotor areas, and these may be differentially sensitive to contextual aspects of the task such as the stimulus properties (di Pellegrino *et al.*, 1992). Alternatively, the more rostral change in the SMA for the spatial SRT learning could be related to the known somatotopy of this area, with the spatial task emphasizing the learning of eye movements and the colour task finger movements (Fried *et al.*, 1991).

Hikosaka *et al.* (1995, 1996) suggest that SMA includes two subdivisions, and that the more anterior region, pre-SMA may be especially important for sequence learning. Several factors should be considered when comparing these conclusions with those of the present experiment. First, learning in the Hikosaka studies occurred without a distracter task and more closely resembled our single-task condition. Their studies did not image other parts of the brain, so any other regions that were acting in concert with the pre-SMA during their experiments could not be determined. Furthermore, the motor component of the task of Hikosaka *et al.* (1995, 1996) is considerably more difficult than that of the SRT tasks. It could be that the pre-SMA is recruited

because of the greater spatial complexity or because of the multijoint responses. Finally, the task used by Hikosaka *et al*. (1995, 1996) requires eye movements. The same is true in the spatial version of the SRT task. There, the SMA activation was somewhat anterior to that in the colour version.

The effects of stimulus characteristics were even more striking in the comparison of the conditions allowing explicit learning. In particular, there was a pronounced shift in the ventral direction when the sequences were cued by stimulus colour. This shift was apparent in both posterior and anterior cortical regions: in the former, the ventral shift was seen near the parietal/occipital border and in the temporal lobe; in the latter, the ventral shift was apparent in prefrontal cortex. Note that there is an even more dramatic dorsal-ventral shift between the implicit and explicit conditions. We restrict our use of the dorsal-ventral terminology to the more subtle differences in the spatial and colour single-task conditions to avoid confusion.

When explicit memory systems are available for learning, significant changes were seen in prefrontal areas associated with working memory, a putative component of explicit sequence learning (Baddeley, 1992). The prefrontal focus was more ventral when the responses were cued by stimulus colour than when the same responses were cued by stimulus position, suggesting that the exact foci of activity within working memory depend on stimulus characteristics. This hypothesis has been proposed by Goldman-Rakic and colleagues for prefrontal function during discrete responses (in Wilson *et al.*, 1993). They recorded from single cells and uncovered a double dissociation to link the ventral and dorsal areas to object and spatial working memory, respectively. When trained monkeys were required to remember the stimulus location, cells in the dorsolateral prefrontal cortex were active during the delay between the presentation of the stimulus and the response, but at baseline when the stimulus pattern had to be remembered. Conversely, cells in the inferior convexity became more active when the pattern had to be remembered and at baseline when location was relevant. Although we used colour rather than shape, our neuroimaging results provide additional evidence that the exact loci within working memory is dependent on stimulus characteristics.

Alternatively, Jonides and colleagues (in Smith *et al.*, 1995) found activation switching from the right to the left hemisphere as the relevant stimulus dimension changed from spatial to object properties. The laterality effect reported in Smith *et al.* (1995) was observed with PET in a working memory task, where subjects had to either remember the location or the identity of stimuli. While this hypothesis and the one advanced by Goldman-Rakic are not mutually exclusive, no such laterality effect emerged when we substituted colours for spatial locations. The primary foci remained in the right hemisphere in both studies.

There are many important differences between the working memory task of Smith *et al.* (1995) and that of the SRT experiments which could result in the varying laterality effects. For instance, all of the stimuli in the working memory task were monochromatic and differed in terms of shape, whereas all the stimuli in the SRT task were identical in terms of shape and differed only in terms of colour. Additionally, increases in rCBF may have remained in the right hemisphere when the task-relevant stimulus characteristic in the SRT task switched to colour because of the persisting spatial quality of the response. That is, the SRT task has a spatial component which persists in colour versions of the task since subjects are still responding by pressing keys which are placed in different physical locations. This property may induce the right hemisphere learning-related increases in rCBF. While it is possible that a laterality effect would have been obtained had we used shape instead of colour, it is plausible that the right hemisphere plays a critical role in explicit learning. On the other hand, the prefrontal activity in the right hemisphere may reflect a critical role for this area in memory retrieval (Kapur *et al.*, 1995). Retrieval would be essential in our spatial and colour sequencing studies given that many of our subjects anticipated the next stimulus.

Another important difference between our studies and those of Smith *et al*. (1995) involves the statistical procedure identifying regions of increased activation. Smith *et al.* (1995) used a subtractive procedure to identify areas of increased rCBF. They performed subtraction across the two tasks, whereas in the SRT experiments, activation was compared within the spatial or colour tasks. When the analogous comparisons are made between the SRT experiments, as in Table 3, the results suggest a similar laterality effect.

Neural systems for motor learning

As in our previous study, sequence learning under dual-task conditions, where attention was diverted from the SRT task, occurred in motor effector regions, including the sensorimotor cortex, SMA and the basal ganglia. Thus, the changes observed with implicit sequence acquisition were independent of stimulus features, suggesting these areas are encoding representations of particular movements. Changes in the sensorimotor cortex have been observed in many functional imaging studies of procedural learning tasks requiring extensive practice (Lang *et al.*, 1988; Grafton *et al*., 1992; Grafton *et al*., 1994; Schlaug *et al*., 1994), including the spatial version of the SRT task (Grafton *et al.*, 1995).

The localization in the human SMA parallels the discovery, in monkeys, of SMA neurons encoding sequences of discrete movements (Mushiake *et al*., 1990; Aizawa *et al*., 1991). Based on the performance of patients with SMA lesions, Halsband *et al*. (1993) have also proposed an important role for the SMA in sequential movements.

In both the colour and spatial SRT studies, SMA activation in the dual-task condition was replaced by premotor activation when the distractor task was removed. These results hold an interesting correspondence with another PET study of sequence learning. Jenkins *et al*. (1994) scanned subjects under three conditions: (i) while at rest, (ii) while performing

overlearned sequences and (iii) while learning new sequences. For brevity, we focus on the comparison between the latter two conditions. The overlearned sequences elicited greater levels of activation in the SMA, while learning new sequences elicited greater activation in the prefrontal and lateral premotor cortex. This pattern of results fits with those obtained in the SRT studies if we assume that their new learning task was similar to our explicit learning condition and the overlearned sequence task was similar to our implicit learning condition. When learning a new task in the Jenkins *et al.* (1994) study, subjects had to form working hypotheses concerning the sequence to be mastered, and had to use feedback information explicitly to modify these hypotheses. With practice, the subjects continued, of course, to have full knowledge of the sequence. However, their performance became more automated with a reduced need to attend to the stimulus–response patterns and error-related information.

An alternative functional distinction between the SMA and premotor area is that the former is essential for internally generated movements, whereas the latter is more important for externally guided movements (Goldberg, 1985; Rizzolatti *et al*., 1983; Passingham *et al*., 1989). At first glance, the current results do not appear to support this scheme. One would assume that single-task performance was more closely linked to internally generated movements since subjects, at least those with explicit awareness, could anticipate the forthcoming response. Under dual-task conditions, the responses were clearly triggered by the appearance of the different coloured stimuli.

One way to reconcile this result with the previous studies is to assume that, in the SMA, movements are organized in terms of successive response elements or internal states. The finding that SMA activation is independent of stimulus characteristics fits nicely with this proposal. Here, motor programmes may be made accessible only by particular stimuli or contexts, but once initiated, they are executed at this level, without reference to the environment. Hence this area shows increased rCBF for both spatial and colour stimuli.

When the next stimulus can be anticipated, as in single-task conditions, it is the premotor area that shows an increase in rCBF. The activation of premotor cortex is accompanied by activation in regions which appear to be stimulus specific. Under single-task conditions, the movements may be organized in relation to the stimuli or representations in working memory (i.e. in the frontal areas) and recruit an entirely separate neural system. Two other PET studies, in addition to the spatial version of the current one, have found activation in the premotor area during early explicit learning of motor sequences (Seitz *et al*., 1990; Jenkins *et al*., 1994).

The SMA, sensorimotor cortex and striatum form a cortical–subcortical motor loop regulating voluntary movement (Alexander *et al*., 1990). Like the SMA, the basal ganglia showed consistent activation under implicit learning conditions for both experiments. Rauch *et al*. (1995) also found increased activation in this region during SRT-sequence learning with PET. This finding is consistent with animal research, emphasizing the importance of the striatum in sequential motor behaviours (Berridge and Whishaw, 1992) and in learning fixed contingencies between environmental contexts and responses (Packard *et al.*, 1989). Furthermore, Kermadi *et al*. (1993) made single-unit recordings from the monkey caudate nucleus while the animal learned to make a sequence of responses. They found cells that were selective not only for particular items but also for the item's sequential context, i.e. some cells responded during the performance of element '3' only when it occurred in the sequence '1→3→2'. These result extend findings of Hikosaka *et al*., (1989), who found cells in the caudate sensitive to the context of remembered saccades.

As is common, studies of patient populations present a less clear picture. Attempts to isolate the specific deficits of individuals with motor structure lesions have been undermined by the general slowing of reaction times and variable performance, making their interpretation difficult. Some research, though, corresponds nicely with the current data. Many clinical studies have found implicit learning deficits in patients with damage to the basal ganglia (Heindel *et al*., 1988; Knopman and Nissen, 1991; Agostino *et al*., 1992; Jahanshahi *et al*., 1992; Pascual-Leone *et al*., 1993). Pascual-Leone *et al*. (1993), for example, concluded that patients with Parkinson's disease were impaired on implicit measures of learning for the SRT and Knopman and Nissen (1991) reported similar findings with Huntington's disease patients.

Another patient population thought to have motor learning deficits are individuals with cerebellar dysfunction. Pascual-Leone *et al.* (1993) reported a severe type of learning deficit in patients with cerebellar lesions. We observed decreased activation in regions of the cerebellum during the sequence blocks, although the foci were quite distinct in the dual and single-task conditions. A decrease in cerebellar activation has been reported in other PET studies following sequence learning (e.g. Jenkins *et al.*, 1994). One explanation is that the cerebellum, operating as an error-detector, compares expectancies with actual movements. Thus, as learning proceeds and expectations increasingly match the required movements, less cerebellar processing is engaged. The actual representation of the sequences and concomitant increases in brain activity occur elsewhere.

Cognitive issues

Recently, some behavioural studies have addressed the computational nature of the representation learned during the SRT task. Keele *et al*. (1995) found near-perfect transfer from one set of effectors to another for learning in the SRT task. In their experiments, some subjects used four fingers while keeping the hand still and others used a single finger and moved the entire arm to respond. When subjects switched modes of responding, a reaction time advantage after having performed sequenced blocks was observed relative to controls

who had performed random blocks. This advantage was maintained even when subjects were asked to count tones simultaneously, which presumably prevented any awareness of the sequence from emerging. From these results, Keele *et al*. (1995) conclude that sequential knowledge is more abstract than a series of muscle movements.

An important aspect of these studies to bear in mind is that responses in the SRT task are typically spatially cued, unlike the present experiment. While subjects perform the task, their eyes presumably move from one stimulus location to the next. Therefore, it is possible that a sequence of eye movements is, at least, part of what is learned in a standard SRT task experiment. If eye movements were, in fact, responsible for improvements in reaction time, then perfect transfer would be expected in the Keele *et al*. (1995) experiments.

Willingham *et al*. (1989) addressed this problem by dissociating cue location from the required response. In their version of the task, subjects were required to make their response based on the colour of the stimulus rather than its location. The stimuli still occurred in different spatial positions, but during training blocks, these locations were independent of the appropriate response. Subjects were then transferred to a more standard version of the task. Then, all the stimuli were the same colour and subjects were asked to respond on the basis of stimulus position. The exposure to the colour sequence showed no benefit of transfer to this new task compared with the all random controls, even though the finger movements made were the same as in the previous blocks where colour determined the response. The authors concluded that learning is not restricted to either perceptual or response systems. Rather, learning incorporates the association of stimulus–response contingencies. This conclusion is intriguing given the frequent assertion that implicit knowledge is inflexible and heavily dependent on context (Schacter, 1992).

Mayr (1996) followed up on the proposal of Willingham *et al*. (1989), that separate neural systems may be involved with the learning of spatial and symbolic sequences; as in the earlier study, Mayr asked subjects to respond according to the identity of symbols which were presented in one of four locations. However, the stimuli in these experiments were more difficult to discriminate to ensure that eye movements would facilitate response selection. For separate experimental groups, the location, the symbol or both aspects of the stimuli were determined sequentially. Under these conditions, learning for the spatial and symbolic sequences appeared to coexist. That is, disruption in either sequence caused increases in reaction time. However, learning of one sequence seemed unaffected by the learning of the other; the group that simultaneously learned both sequences showed equivalent costs and benefits to groups that learned only a single sequence. This pattern of independence led Mayr (1996) to conclude that separate systems may be involved in the implicit acquisition of nonspatial and spatial sequences. The differences we observed in the parietal cortex and SMA, between the spatial and colour implicit conditions, may provide the neural mechanisms for independent, stimulus-specific learning modules.

One apparent paradox presented by the spatial and colour experiments involves the common neural loci for implicit learning contrasting with the separate neural loci for explicit learning. Willingham *et al*. (1989) found no evidence of transfer of learning without explicit knowledge when the same sequence of responses was required in the location condition as in the colour condition. Preliminary work in our lab has also shown little evidence for transfer of learning in the SRT when the sequence is acquired with one set of stimuli and then tested using a different set. Presumably, if sequence knowledge becomes explicit, subjects should be able to apply the knowledge regardless of the specific stimuli being employed. Yet, the present PET data would suggest the opposite pattern of results.

The literature suggests two possible resolutions to this paradox. First, the lack of transfer fits nicely with the characterization of implicit knowledge as ‘inflexible’ and ‘context dependent’ that pervades the memory literature (e.g. Packard *et al*., 1989; Tulving and Schacter, 1990; Schacter, 1992; Hikosaka, 1993). However, this property is somewhat dissonant with the convergence of neural loci identified in the dual-task versions of the spatial and colour tasks. Several possible explanations exist. First, the implicit system could be more densely packed in the brain, so that the spatial resolution provided by PET is unable to distinguish what are in fact different regions, i.e. the separate stimulus-dependent regions may be physically closer to each other in the implicit system than in the explicit system, so much so, that they appear to be a single region. In fact, the identified regions in the two versions of the tasks were slightly different. This explanation finds support in the behavioural study by Mayr (1996), who concluded that implicit learning could take place independently for spatial and non-spatial modalities.

A second hypothesis involves the potential role of the common regions. These areas may encode sequential information in reference to specific stimuli or environmental cues. Even if the encoded information is organized as a series of movements, it may be accessible only in a particular environmental context. The stimulus-specific aspect driving the sequential knowledge may therefore involve systems that are computationally upstream from the expression of striatal and SMA activity. Though these two structures may be playing an identical role in the two experiments, the function cannot be accessed unless the contexts are the same. In the dual-task conditions there were indeed areas of increased activation that were not shared by the two versions of the experiment. For the spatial version of the task, such regions included the left anterior frontal, left parietal and left lingual gyrus. For the colour version, non-overlapping increases in rCBF were found in the inferior parietal, parietal operculum areas, in both the left and right hemispheres. These areas could represent the context-dependent aspects, feeding into the common set of regions (such as the SMA and basal

ganglia) responsible for the sequential organization of responses.

Summary

Stimulus characteristics have different effects on the neural mechanisms associated with implicit and explicit sequence learning. For the implicit system, stimulus characteristics had a minimal effect on the neural locus of motor learning; the spatial and colour versions of the tasks indicated largely overlapping regions in the contralateral hemisphere when subjects were concurrently performing a tone counting tasks. The SMA and basal ganglia appear to be important for context-dependent motor-associative learning. Further work is required to determine the significance of response properties on the recruitment of these structures.

In contrast, the neural correlates of explicit learning and memory were highly dependent on stimulus characteristics. Although learning without distraction of either spatial or colour sequences activated more ventral regions than in the dual-task condition, there was an additional ventral shift for the colour sequences. This shift was apparent in the frontal, temporal and parietal/occipital cortex. The frontal shift is most intriguing, providing the first PET evidence for the notion of a dorsal/ventral dichotomy within the working memory system posited by Goldman-Rakic and colleagues in Wilson *et al*. (1993).

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