RESEARCH ARTICLE

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Neural correlates of encoding and expression in implicit sequence learning

Abstract In the domain of motor learning it has been difficult to separate the neural substrate of encoding from that of change in performance. Consequently, it has not been clear whether motor effector areas participate in learning or merely modulate changes in performance. Here, using a variant of the serial reaction time task that dissociated these two factors, we report that encoding during procedural motor learning does engage cortical motor areas and can be characterized by distinct early and late encoding phases. The highest correlation between activation and subsequent changes in motor performance was seen in the motor cortex during early encoding, and in the basal ganglia during the late encoding phase. Our results show that rapid encoding during procedural motor learning involves several distinct processes, and is represented primarily within motor system structures.

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Introduction

The study of learning mechanisms is hampered by the fact that learning itself cannot be measured; rather, learning is inferred to have occurred when there are lasting changes in performance. The problem of disentangling learning from performance becomes especially important for investigating the neural substrates of motor learning, because changes in motor performance alone frequently lead to changes in neural activity in areas also thought to be involved in learning. For example, cerebellar activity tracks motor error (Kitazawa et al. 1998), and activity in frontal (Mattay and Weinberger 1999; Jancke et al. 1998; Deiber et al. 1999; Turner et al. 1998) and sub-cortical (Turner et al. 1998) motor areas increases with the rate of movement. Recent implicit learning data suggest, however, that there are paradigms capable of temporally dissociating motor learning from its expression (Seidler et al. 2002).

Implicit learning is characterized by a lack of awareness of the learning process and its content (Cleeremans 1993; Reber 1993). Several investigators have used the serial reaction time (SRT) task to study implicit learning (Nissen and Bullemer 1987; Grafton et al. 1998; Willingham et al. 1997). Typically, in the SRT task, subjects respond to one of four illuminated display boxes by pressing the corresponding button on a keypad. When a complex sequence of visual stimuli is presented unbeknownst to the subject, the response time (RT) of the associated finger movement decreases compared with that seen for random stimuli; this improvement in response time is an index of learning. Several

SRT experiments have required subjects to perform a concurrent visual or auditory attention-distracting task (Seidler et al. 2002; Nissen and Bullemer 1987; Frensch et al. 1999). The effects of this secondary task may vary (Shanks and Channon 2002; Frensch et al. 1998, 1999; Hsiao and Reber 2001; Jimenez and Mendez 1999), but can include (1) blocking the explicit awareness of learning when the sequence is relatively short, or, under some conditions, (2) the apparent inhibition of the implicit learning itself. In the latter case, there may be a true inhibition of learning or merely a failure to express the learning, depending on the distractor conditions; these possibilities may be distinguished by testing subjects in a transfer task following removal of the distractor (Seidler et al. 2002; Frensch et al. 1998, 1999). There are other behavioral manipulations, such as increasing the variance or the duration of the response-to-stimulus interval that may also affect expression of learning (Willingham et al. 1997). In summary, although there are a variety of methods whereby the process of sequence encoding may be temporally dissociated from change in performance during SRT tasks, the use of specific distractors provides a unique opportunity to do so without disrupting the dynamics of the task itself. In the current study, we use a visual distractor that effectively dissociates learning from performance (Seidler et al 2002).

Although there is no clear unanimity as to the substrate of implicit motor learning (Doyon et al. 1996; Rauch et al. 1997), the majority of studies have identified a network of common areas that subserve this process which include motor cortex, supplementary motor area (SMA), putamen, and inferior parietal cortex (Grafton et al. 1995, 1998; Pascual-Leone et al. 1994; Hazeltine et al. 1997; Honda et al. 1998). In these studies, the learning-associated areas were identified through either a general (Grafton et al. 1995, 1998; Hazeltine et al. 1997) or a specific parametric correlation with performance changes (Sakai et al. 1998). Consequently, the question remains whether the network of areas, identified for implicit motor learning, is involved in modulating performance, learning, or both. Furthermore, if the network is involved in learning per se, the underlying neural time course remains to be elucidated. These questions are the focus of the current work.

Materials and methods

Subjects

Two groups of right-handed adults participated in this study, ranging in age from 23 to 38 years. The experimental group consisted of six females and five males; the control group consisted of six females and three males. All subjects signed a consent form approved by the Institutional Review Board and filled out a health history questionnaire.

Experimental setup and procedure

The experimental subjects lay supine in a 4.0 T MRI scanner. Stimuli were projected onto a screen that was visible to the subjects in an adjustable mirror attached to the volume coil (Vaughan et al. 2001). Subjects made responses on a key-press device with the fingers of the right hand. There was a separate response button and corresponding visual stimulus box for each finger. The subjects were instructed to press the appropriate button as fast as possible when an "X" appeared in one of the stimulus boxes. For some trial blocks, the stimuli were presented in a repeating fashion and for others a pseudorandom presentation was used. Each subject was presented with a 12-element sequence, in which each of the four possible stimulus locations was presented three times (Willingham et al. 1997). In order to prevent explicit awareness, the sequence was constrained such that a stimulus could not be presented twice in a row, there could be no runs of four (e.g., 1234), and there could be no trills of four (e.g., 2424). If subjects did not respond by pushing the correct button, then the same stimulus location was presented again on the next trial. Subjects were not informed about the presence of the sequence. Each block consisted of 94 trials, spaced by a constant inter-stimulus interval of 800 ms. Each time the sequence was repeated, the presentation would start at a different random point within the sequence with the additional criterion that the sequence requirements continued to be met. The random blocks were made up of different 12-element sequences appended together.

Subjects performed a concurrent distractor task during some of the blocks. This task required subjects to watch a square placed centrally above the other stimuli. The square changed color to one of four possible colors at a rate of 3 Hz. Subjects were instructed to watch for a target color and to keep track of the number of times it appeared within one block, providing a verbal report after each block. We stopped fMRI acquisition after each block for approximately 2 min, allowing subjects to make the verbal report and providing them with a brief break. The target color was presented at a rate of 1–3% (i.e., 1–3% of color presentations occurring at 3 Hz during a 75-s task period = 2–7 color targets per block), while the other three colors were equally distributed. Blocks 1–6 comprised the encoding phase of the experiment and were all presented with a concurrent distractor. Random stimuli were presented during blocks 1 and 6, while the sequence was presented in blocks 2–5. Blocks 7–9 comprised the expression phase of the experiment and were performed without the distractor task. Blocks 7 and 9 were random; block 8 was sequence. The median response time was computed for each subject for each repetition of the sequence. Then a mean response time across repetitions was computed for each subject within each block. These mean values were averaged across subjects for presentation purposes.

Subjects were tested for explicit awareness of the sequence at the end of the experiment. They were first asked whether they had noticed anything about the manner in which the finger movement stimuli were presented. They were then informed that there was a sequential presentation for some of the blocks, and were asked to perform a free recall of the sequence. Rather than responding to a stimulus, however, the stimuli were presented as feedback each time that they pressed a button. Subjects reporting greater than five stimuli (in a minimum run of three elements) correct were considered to have explicit awareness of the sequence (cf. Willingham et al. 1997).

We also tested a group of control subjects in a purely behavioral experiment outside of the scanner. In this variant of the SRT task with distractor, the "encoding" phase comprised blocks 1–6, during which all the stimuli were presented randomly in the presence of the distractor. The expression phase, as in the imaging experiment, comprised blocks 7–9 without distractor; blocks 7 and 9 were random and the sequence was presented in block 8. The data from this control experiment allowed us to compare the response time savings at expression of learning for subjects that had previously practiced the sequence with those that had not.

Image acquisition

Images were acquired in a 4 Tesla magnet (Siemans, Erlangen, Germany and Varian, Palo Alto, Calif., USA). Functional images were acquired using a single-shot gradient-echo EPI [field of view 220×220 mm, voxel size 3.4×3.4×5 mm, TR = 3.4 s (or 3.38 s, depending on the number of slices acquired), TE = 35 ms]. In five subjects, we acquired 17 slices encompassing the entire brain except for the cerebellum. In the remaining six subjects, the cerebellum was recorded as well for a total of 26 slices. Our SPM analyses were computed over regions of the brain that were imaged for all of the subjects. Therefore, only the 17 upper slices were analyzed for the current study. The results for the cerebellum (slices 18–26 in six of the current subjects) are described in full in Seidler et al. (2002). A total of 34 volumes were collected for each block. The first ten volumes comprised a control period during which subjects simply watched the static stimulus boxes without moving. The task began on the 11th volume and was presented for 75.2 s (94 trials with 800-ms intertrial intervals). We analyzed 22 volumes as being task related (74.8 s). We collected an additional two volumes, but did not analyze them. The first three scans were omitted from each run, in order to allow the MRI signal to reach its steady state. This resulted in seven control and 22 task volumes for each block. Structural images were acquired using FLASH (field of view 240×240 mm, voxel size 0.86×0.86×5 mm, TE = 4.3 ms, either 26 or 17 slices).

Data processing

The images were first converted to Analyze format for analysis with SPM99 (Wellcome Department of Cognitive Neurology, London, UK; see Friston et al. 1995) in the Matlab environment (Mathworks, Sherborn, Mass., USA). We performed head motion corrections and examined the results to ensure that subjects did not move more than two millimeters during the experiment. A mean functional image for each subject was also computed at this time. The structural image was then co-registered to this mean image and spatially normalized to the Montreal Neurological Institute (MNI) template (Evans et al. 1994). The obtained normalization parameters were then applied to the subject's functional images. The functional images were smoothed with a Gaussian smoothing kernel with a full width at half maximum of 6 mm.

The imaging data were analyzed in two parts. The first part, aimed at determining brain regions involved with implicit sequence encoding, was performed over the first six blocks (one random block, four sequence blocks, one random block). The models were created as boxcar functions time locked to the effect of interest and convolved with an estimate of the hemodynamic response function. To assess sequence encoding, an activation map was first computed to reveal the voxels that showed greater task activation (finger tapping versus passive rest) for the sequence blocks than for the two bounding random blocks. For each block, we used just the passive rest period preceding the block being analyzed as opposed to all of the baseline periods. This was necessary because we stopped the fMRI acquisition between each block, as described above. This mask was applied using a $P < 0.005$, uncorrected. Then, we performed two different contrasts created to capture the temporal dynamics of learning, and masked them by the encoding activation map. The analysis identified all voxels that exhibited activity consistent with the hypothesized models. One model searched for activation that was initially high for the encoding period compared to the initial random block that then progressively decreased across the remaining sequence blocks in a linear fashion. This model reflects processes that are active earlier in learning. The second model looked for longitudinally increasing activation across the four sequence blocks. This model reflected processes that were increasingly active later in learning. Each of these models was first run on the individual subject data. Then, these individual subject contrast images were combined into two SnPM (Nichols and Holmes 2002) analyses. We performed an additional SnPM analysis for the sequence greater than random contrast (constant activation) to also identify regions related to encoding that may not have shown time-varying changes in activation. For all three SnPM analyses, we employed a multiple subject design with one scan per subject (the resulting early learning, late learning, or constant learning contrast image for each subject), with 10 mm variance smoothing

and 1,000 permutations. The results were evaluated at a corrected $P < 0.05$ level, with a spatial threshold of ten contiguous voxels.

Since there were no significant changes in RT occurring across the sequence blocks, it was meaningless to correlate the individual subject's RTs with differences in intensity of activation. As an exploratory analysis, however, we examined the relationship between an individual's RT difference at expression of learning; that is, the RT of block 8 subtracted from the average RT of blocks 7 and 9, and the activation changes that were occurring during sequence encoding. These correlations were evaluated for significance using a threshold of $P < 0.05$ (R critical $= 0.58$). We compared the correlations in motor cortex during two epochs, encoding and expression, by converting the R for each case into a Fisher's Z score and then testing for significance.

Expression of learning was examined by comparing sequence block 8 to random blocks 7 and 9 with a simple contrast, masked with an activation contrast (finger tapping vs. passive rest) performed across the three blocks. Single subject data were combined into a group analysis, using the SnPM design described above. RT differences for individual subjects were also correlated with individual changes in intensity masked with the activation contrast across blocks 7–9. Finally, we performed conjunction analyses to determine whether there was any significant overlap between the regions contributing to either early, late, or constant encoding and expression of learning. These analyses were evaluated at the $P < 0.005$ (uncorrected) level, with a spatial threshold of ten contiguous voxels.

We converted the activation coordinates from MNI space to the Talairach atlas (Talairach and Tournoux 1988) for functional localization (see http://www.mrc-cbu.cam.ac.uk/Imaging/). Significant areas of activation were then localized using this atlas, with medial motor areas identified as in Picard and Strick (1996). The time course of activation was plotted for illustrative purposes. The time course was computed as a difference score for each block with the mean magnitude of activation during the control period subtracted from the mean magnitude of activation during the task period. This computation was performed for the single voxel exhibiting the peak of activation in each cluster.

Finally, the RT and error data were analyzed using a repeated measures ANOVA, with repeated measures on block. Repeated contrasts were used as the follow-up test, comparing each block to its preceding one to determine where the differences lay.

Results

Behavioral data

One of the experimental subjects acquired extensive explicit knowledge of the sequence and was able to reproduce eight of the 12 elements correctly. Therefore,

her data were omitted from further analyses. The control group and the remaining ten experimental subjects reported correctly 3.4 elements out of the 12-element sequence. The number of errors that subjects made on the secondary distractor task ranged on average from 0 to 0.3 errors per block (difference between the number of targets presented and the number of targets reported).

There was no change in RT or in the number of tapping errors made during the encoding phase of the study in either the experimental or control group of subjects. Figure 1a plots RT across the nine blocks for both groups. The repeated contrasts resulted in a group × block interaction for block 7 versus block 8 ($F_{1,17} = 20.8$, $P < 0.01$) and block 8 versus block 9 ($F_{1,17} = 7.9$, $P = 0.01$), with no other comparisons achieving significance. The error data are presented in Fig. 1b. There were no group × block effects for the error data. The repeated contrasts revealed a significant difference between blocks 8 and 9 ($F_{1,17} = 5.9$, $P < 0.05$).
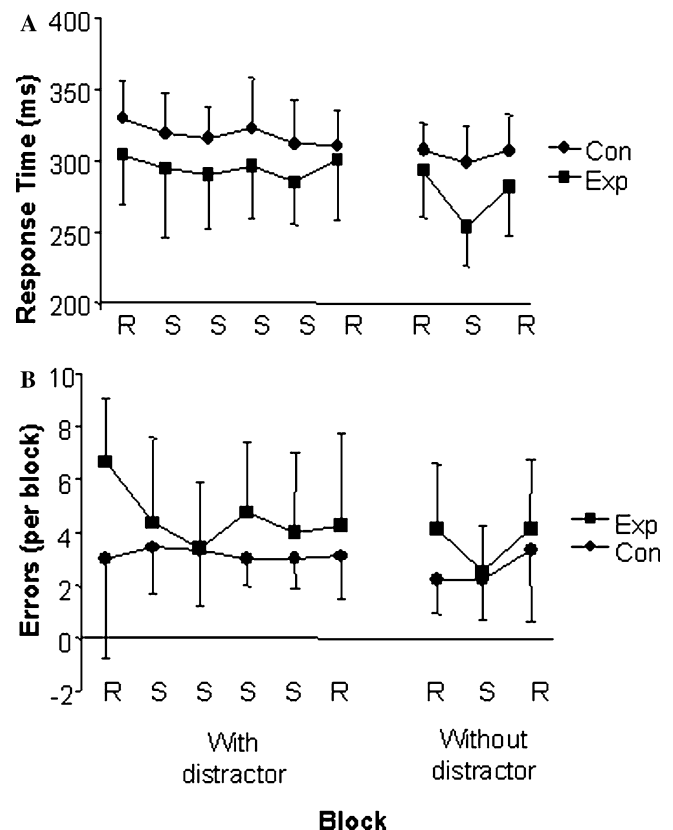

Fig. 1a, b Behavioral results. **a** The mean and standard deviation of response time across blocks for the experimental (*Exp*) and control (*Con*) subject groups. There were no significant changes in response time across the blocks in which subjects performed the concurrent distractor task (blocks 1–6). Response time for block 8 (*sequence*) was significantly less than the response times for blocks 7 and 9 (*random*) for the experimental but not the control subjects. **b** The mean and standard deviation of the number of errors subjects made. Similar to response time, there were no significant differences across blocks 1–6. There were no group × block interaction effects at expression of learning

fMRI data: activation during sequence encoding

Early activation

Early in learning, motor and temporal cortical regions showed significant activity. This activation declined back to the level seen in the first random block by the last sequence encoding block. The motor areas included the left primary motor cortex and the cingulate motor area. This early learning activation is overlaid onto select anatomical slices in Fig. 2, with the time course of activation shown in the lower portion of the figure and the coordinates of local maxima presented in Table 1.

Late activation

The regions showing significant activation later in learning included the anterior cingulate cortex, temporal and occipital cortical regions, and the left basal ganglia. The late learning results are shown overlaid onto anatomical slices in Fig. 3, with the time course of activation presented in the lower portion of the figure and the coordinates of local maxima presented in Table 1.

Constant activation

The only region identified as contributing to encoding in a constant manner was the right inferior parietal lobule. The coordinates for the local maximum of this activation are presented in Table 1.

Correlations with response time

Correlations between the intensity of activation of the areas activated during encoding and the observed RT difference at expression were computed to determine which areas were the best predictors of the magnitude of learning for individual subjects. As indicated in Table 1, the left primary motor cortex and the left basal ganglia exhibited activation that correlated with the eventual RT savings seen at expression. Scatter plots of these relationships are shown in the lower portions of Figs. 2, 3.

fMRI data: expression of learning

Areas contributing to the expression of learning included prefrontal cortex (R middle frontal gyrus), pri-

▶

mary motor cortex, supplementary motor area, anterior cingulate and motor cingulate areas, and inferior parietal lobule (Fig. 4). Coordinates of local maxima are presented in Table 2.

Correlations were computed between the extent of activation in the areas recruited during expression and
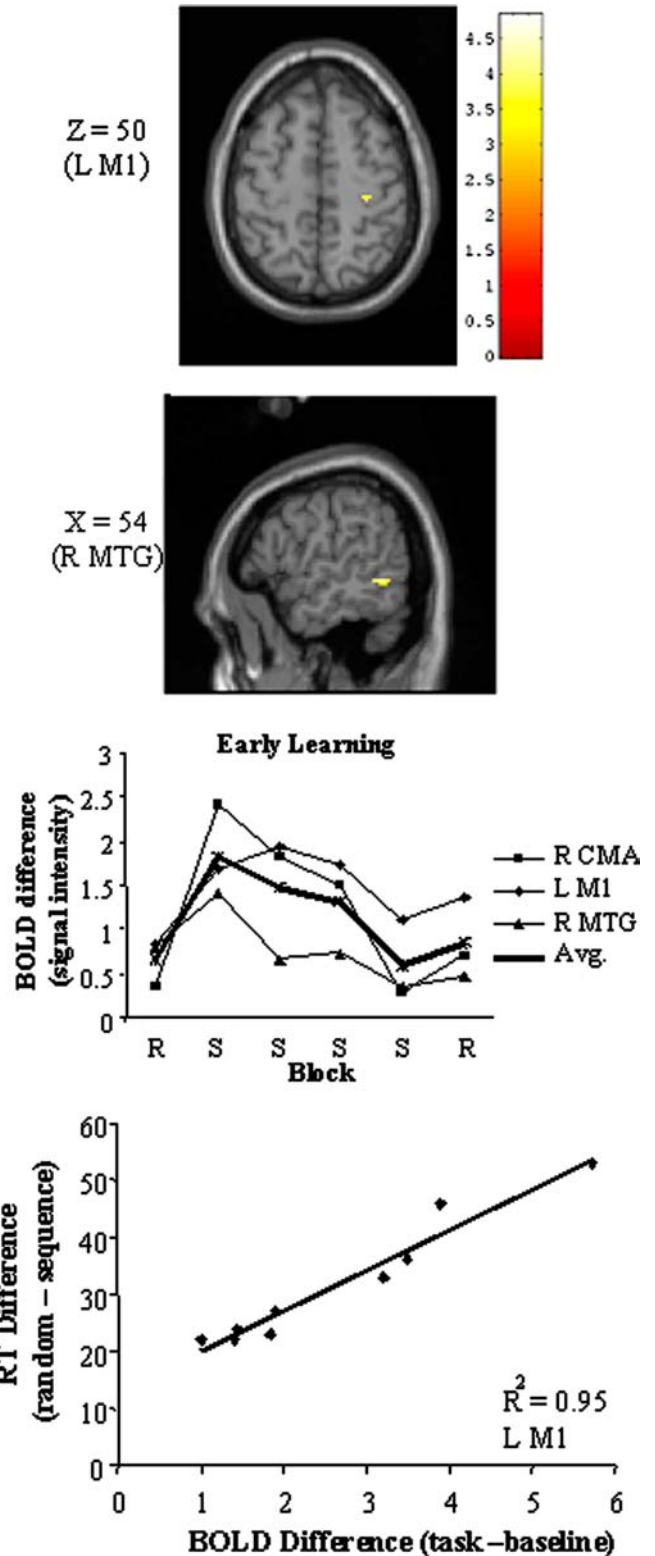

Fig. 2 Early learning activation. Sample images of activation during early encoding. Right in the figure corresponds to the subjects' left. The images depict activation in the left primary motor cortex (*axial slice*) and the right middle temporal gyrus (*parasagittal slice*). The time courses of activity for regions participating in early learning processes are presented in the panel below these slices. *R CMA* right cingulate motor area; *L M1* left primary motor cortex; *R MTG* right middle temporal gyrus; *Avg.* average across the three regions. The *lowest panel* plots the relationship between early learning activation in the left M1 and the eventual expression of learning that individual subjects show (RT difference between random and sequence blocks at expression)

Table 1 Areas active during the encoding phase of sequence learning. The R^2 describes the relationship across subjects between activation during the first sequence block and the eventual RT savings observed at expression (*CG* cingulate gyrus, *PG* precentral gyrus, *MTG* middle temporal gyrus, *AC* anterior cingulate, *STG* superior temporal gyrus, *OG* occipital gyrus, *CUN* cuneus, *PUT* putamen, *IPL* inferior parietal lobule)

	Coordinates	Pseudo T	P	No. of voxels	R^2
Early learning areas					
R CG	2, −22, 25	4.30	0.016	12	0.05
L PG (M1)	−30, −13, 45	4.22	0.016	14	0.95*
R MTG	55, −51, −1	4.12	0.016	11	0.14
Late learning areas					
R AC	2, 39, 33	4.56	0.016	83	0.02
R STG	61, −11, 6	3.52	0.031	14	0.06
R OG	18, −66, 1	3.43	0.047	17	0.19
R CUN	4, −46, 8	3.40	0.047	13	0.19
L PUT	−26, 10, −4	3.24	0.047	11	0.72*
Constant learning areas					
R IPL	57, −52, 41	4.5	0.016	36	0.45*

*R^2 values that were significant at $P < 0.05$

the RT difference observed for each subject. Significant correlations were present for supplementary motor area, anterior cingulate and middle frontal gyrus as indicated in Table 2. Surprisingly, the motor cortex activity was not significantly correlated with RT change at expression.

The conjunction analyses revealed that there was no overlap between the regions contributing to early, late, or constant encoding and expression of learning.

Discussion

In the current study, we effectively dissociated learning from performance change during the SRT task. The experimental subjects exhibited a significant response time savings at expression of learning while the control subjects did not. The error data supported that this effect was not due to a speed-accuracy tradeoff. This dissociation of learning from performance change enabled us not only to separately identify learning and performance related areas but also to address the issue of how learning is represented in this type of procedural motor learning. The ability to study implicit sequence encoding in the absence of performance changes led to uncovering two distinct encoding networks, each following a unique time course. It should be noted that the activations we documented were distinct from those associated with the purely visuo-motor or general stimulus-response mapping aspects of the task, which had been subtracted by the sequence-random mask.

There has been a great deal of debate regarding what exactly is learned by subjects during the performance of the SRT task (Curran and Keele 1993; Clegg et al. 1998; Willingham et al. 2000, 2002; Heyes and Foster 2002). Despite the lack of change in performance during the encoding phase of this experiment, it is clear from the imaging data that at least three distinct processes related to learning the sequence were being carried out (Sakai et al. 1998). One process was related to learning in a constant fashion. Another was related to early learning, with an initial increase in activation at the beginning of the sequence presentation compared with the random block. This activation decayed across the subsequent three sequence blocks. The third process occurring during sequence encoding was captured by a contrast consisting of a steadily increasing function of activation across the four sequence blocks, reflecting areas contributing to late learning. In interpreting these increasing and decreasing patterns of activation as early and late learning, we are making the assumption that learning was continuous across our training period. Moreover, in this design, we were unable to determine whether encoding was completed by the end of our training phase, so the term "late learning" is relative to our training period as opposed to an absolute measure of encoding. We propose that the different time courses of activation in specific brain areas reflect the types of representation required for learning in the SRT task, and elucidate, based on the sets of areas activated, the several different simultaneous processes which contribute to this representation.

Representation of learning during early encoding

The early learning encoding process activated motor cortex, the middle temporal gyrus and the cingulate motor area. To learn a sequence, the subjects must first be able to detect and encode, even below the level of awareness, a pattern. There has been much debate as to whether this encoding is primarily perceptual or motor (Willingham 1999). Our data suggest that even at the earliest encoding stage, both are important. The middle temporal activation, most accurately localized to BA37, has been associated with both visuo-spatial perception (Malach et al. 1995; Owen et al. 1996a; Wheeler et al. 2000) and encoding spatial location (Goldberg et al. 1996; Lacquaniti et al. 1997; Aguirre and D'Esposito 1997; Owen et al. 1996b, 1998), and might encode an
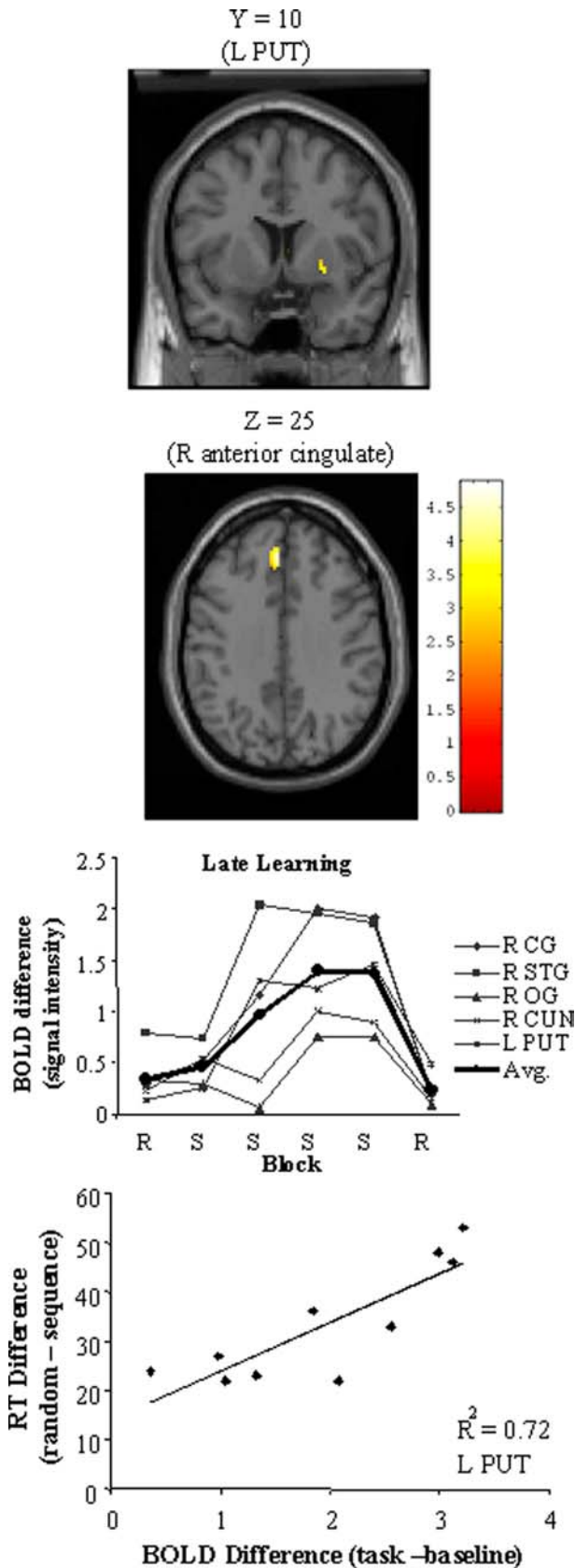
early perceptual representation of the sequence. Although there is adequate behavioral evidence that a perceptual representation is important in procedural learning (Mayr 1996; Remillard 2003), it is unlikely that it is the only representation (Willingham 1999).

We believe that the motor representation in the current experiment was subserved by both motor cortex and motor cingulate. Several aspects of the motor cortex activation were notable. The first was that it occurred during the encoding phase in the absence of an associated change in motor performance. Several previous implicit learning studies have found motor cortex to be involved with the later stages of sequence learning (Hazeltine et al. 1997; Pascual-Leone et al. 1994; Grafton et al. 1998). Some of these investigations did employ dual task paradigms to ensure that learning was implicit (Grafton et al. 1998; Hazeltine et al. 1997); however, there were still concurrent performance changes occurring during learning. These continuing performance changes may have driven the late learning activation in motor cortex, since in the current study we found specifically early activation of this region. While early contributions of M1 to motor learning have been observed previously, subjects either learned the sequence explicitly (Toni et al. 1998; Eliassen et al. 2001), or the time scale over which the learning was assessed covered a much longer time period than in the current study (Karni et al. 1998).

The second notable feature of motor cortex activation in the current study was that the correlation between activation and subsequent measures of learning was very high, even in the absence of performance change ($R^2 = 0.95$). It is important to note that the correlation with learning we documented was seen during encoding but not during expression; this would not have been predicted for a motor effector area such as the motor cortex. Nevertheless, motor cortex has been shown to play a role in many cognitive aspects of motor control beyond the encoding of simple movement parameters (Georgopoulos 2000) and appears to be involved at an early stage in the consolidation of motor skill learning (Muellbacher et al. 2002). We propose that early activation in motor cortex during encoding was the

Table 2 Areas active at expression of learning. The R^2 describes the relationship across subjects between the activation difference between R and S blocks at expression, and the RT savings observed at expression (*MGF* middle frontal gyrus, *SMA* supplementary motor area, *PG* precentral gyrus, *CG* cingulate gyrus, *IPL* inferior parietal lobule, *SOG* superior occipital gyrus)

Area	Coordinates	Pseudo T	P	No. of voxels	R^2
R MFG	26, 48, 20	6.49	0.016	42	0.48*
R SMA	6, −28, 68	6.39	0.016	23	0.08
R SMA	12, −20, 65	4.25	0.016		0.37*
L PG (M1)	−14, −18, 67	6.27	0.016	17	0.09
L CG	−8, −18, 27	5.72	0.016	20	0.21
R CG	−2, 24, 8	5.54	0.016	51	0.51*
L IPL	−48, −62, 42	4.52	0.016	44	0.05
L SOG	−36, −70, 42	4.03	0.016	44	0.01

*R^2 values that were significant at $P < 0.05$

result of a rapid functional re-organization that facilitated the movements involved in the sequence even in the absence of performance change.
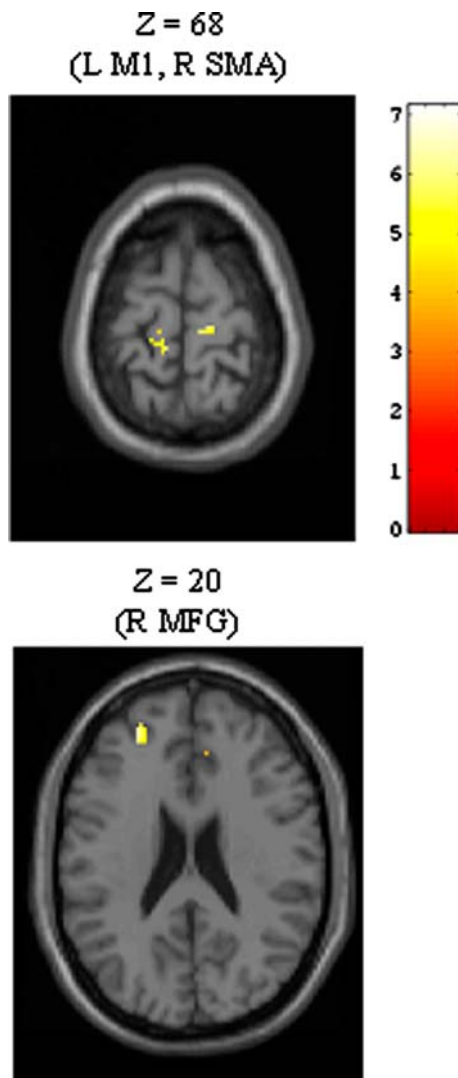

Fig. 4 Expression of learning activation. A few sample slices of the expression of learning activation are presented. *Right* in the figure corresponds to the subjects' left. These axial slices depict activation in the left motor cortex (MI) and the right supplementary motor area (SMA; *upper slice*), and the right middle frontal gyrus (MFG; *lower slice*)

The other component of early encoding was seen in "motor" cingulate, or the caudal cingulate zone (CCZ) in the nomenclature of Picard and Strick (1996). The locus of activation closely conforms to posterior BA24 and has been activated in previous studies of procedural motor learning (Grafton et al. 1998; Eliassen et al. 2001). In many respects, this motor area may be regarded as functionally similar to motor cortex, given its strong cortico-spinal projections. We believe that its activation also reflects an early motor representation of the sequence. In summary, early coding, in the absence of performance change, and following subtraction of the standard visuo-motor aspects of the task, has both a perceptual and motor representation.

Representation of learning during late encoding

The second process occurring during sequence encoding was captured by a contrast consisting of a steadily increasing function of activation across the four sequence blocks. The areas activated included right anterior cingulate, right superior temporal gyrus, in addition to occipital cortex and cuneus. The anterior cingulate (BA32), which had the largest volume of activation, has previously shown activation during both implicit and explicit sequence learning (Grafton et al. 1998; Eliassen et al. 2001; Destrebecqz et al. 2003). This area is also engaged when subjects are exposed to stimuli that violate a previously repeating pattern (Huettel et al. 2002). In the context of the current experiment, we propose that as subjects continue to be exposed to the sequence they increasingly monitor their performance; in other words they are establishing a set of rules or expectations that can be used as a basis for future cognitive control. This is a slight variation of the current concept of anterior cingulate, which is that it is primarily engaged in conflict monitoring (van Veen et al. 2001; Botvinick et al. 1999; Cohen et al. 2000).

The increasing activation over time in superior temporal gyrus is at variance with the results of other studies. Both the left superior temporal gyrus (Wernicke's area) and its right hemisphere homologue have shown decreases in activation over time when subjects observed a spatial sequence of visual stimuli (Bischoff-

Grethe et al. 2000) or performed a predictable sequence of finger movements (Grafton et al. 1995). We suggest that the reason for the differences relates to the extent to which subjects had learned the predictability. For example, Bischoff-Grethe et al. (2000) presented their subjects with a repeating four-element sequence of visual stimuli in nine 90-s blocks, while Grafton et al. (1995) used a six element sequence repeated continuously over eight blocks. Presumably, subjects learned both these sequences early in the experiment. By contrast, in the current experiment we used a 12-element sequence that was presented with a distractor, making it more difficult to learn, over four blocks of trials. We propose that superior temporal cortex is engaged in learning predictability in the environment through increased activation as in the current experiment, but shows decreased activation during repeated rehearsal of familiar information when most of the learning has already taken place. Obviously, this hypothesis remains to be rigorously tested.

The basal ganglia, which have long been regarded as a repository of "motor programs", are active during implicit learning in humans (Rauch et al. 1997; Grafton et al. 1995; Hazeltine et al. 1997), and are important for the control of sequence behavior in animals (Kermadi and Jurquet 1993; Berridge and Whishaw 1992). Although the representation within the basal ganglia is clearly motor, it is not likely to be effector-specific; this would be consistent with behavioral studies using the SRT task (Curran and Keele 1993). We believe that during this "late" stage of learning, the sequence detected early in learning is being encoded in a relatively high level representation that is accessible to the motor system, as evidenced by the high correlation between basal ganglia activation and the subsequent performance change.

Although our primary interest was in processes that changed dynamically during encoding, we documented one brain area, right inferior parietal lobule, that showed consistent activation throughout encoding. There is evidence that inferior parietal cortex is involved in the abstract representation of sequence knowledge (Grafton et al. 1998); it would appear that the brain begins to build this abstract representation as soon as the sequence is presented and the process continues at least as long as the relatively short encoding time examined in the current study.

Expression of learning

Upon removal of the distractor task it was evident that the subjects had learned the sequence. The volume of brain activity associated with the expression of the previously learned sequence was quite large compared with that during any of the encoding contrasts. Moreover, there was no overlap found between the regions contributing to encoding and those engaged at expression of learning, implying that differing regions contribute to

encoding versus recall processes utilized at expression. Activation of the inferior parietal lobule has been observed in many previous studies of motor learning (Grafton et al. 1995, 1998; Hazeltine et al. 1997; Jenkins et al. 1994; Eliassen et al. 2001; Lafleur et al. 2002), and is thought to represent sequence learning in a non effector-dependent manner, which is then translated into an effector-specific representation by motor cortex and perhaps motor cingulate (Grafton et al. 1998). Our data suggest that this brain area is also essential in enabling the learned sequence to be reflected in changes in motor performance.

Our understanding of the role of SMA has evolved over the years to the current consensus that is most likely engaged during the production of previously learned sequences (cf. Tanji 2001; Hikosaka et al. 1999). The current results support this position as SMA does not appear to have any role in learning the SRT task, merely in its execution. Although the dorsolateral prefrontal cortex is typically associated with explicit memory processes (Eliassen et al. 2001; Destrebecqz et al. 2003; Levy and Goldman-Rakic 1999), other studies have shown its involvement in implicit learning in the SRT task (Willingham et al. 2002; Rauch et al. 1997), during the maintenance of contextual information in an investigation of implicit grammar learning (Berns et al. 1997), and during spatial working memory tasks (Jonides et al. 1993; Owen et al. 1996c). It has been suggested that activation of dorsolateral prefrontal cortex during implicit learning in the SRT task is related to a conscious selection of isolated upcoming movements, based on an intuitive knowledge of the sequence, though knowledge of the sequence itself may not have reached the level of awareness (Willingham et al. 2002). Consistent with this hypothesis, it is notable that the activation of prefrontal cortex in the current study only occurred when the subjects were in a position to express their learning. The idea that this brain area may be involved in the selection of response locations based on spatial information is also consistent with the finding that transcranial magnetic stimulation of prefrontal cortex prevents learning in the SRT task, but only when the learning is based on spatial information (Robertson et al. 2001).

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