The Role of the Dorsolateral Prefrontal Cortex during Sequence Learning is Specific for Spatial Information

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Many studies have implicated the dorsolateral prefrontal cortex in the acquisition of skill, including procedural sequence learning. However, the specific role it performs in sequence learning has remained uncertain. This type of skill has been intensively studied using the serial reaction time task. We used three versions of this task: a standard task where the position of the stimulus cued the response; a non-standard task where the color of the stimulus was related to the correct response; and a combined task where both the color and position simultaneously cued the response. We refer to each of these tasks based upon the cues available for guiding learning as position, color and combined tasks. The combined task usually shows an enhancement of skill acquisition, a result of being driven by two simultaneous and congruent cues. Prior to the performance of each of these tasks the function of the dorsolateral prefrontal cortex was disrupted using repetitive transcranial magnetic stimulation. This completely prevented learning within the position task, while sequence learning occurred to a similar extent in both the color and combined tasks. So, following prefrontal stimulation the expected learning enhancement in the combined task was lost, consistent with only a color cue being available to guide sequence learning in the combined task. Neither of these effects was observed following stimulation at the parietal cortex. Hence the critical role played by the dorsolateral prefrontal cortex in sequence learning is related exclusively to spatial cues. We suggest that the dorsolateral prefrontal cortex operates over the short term to retain and manipulate spatial information to allow cortical and subcortical structures to learn a predictable sequence of actions. Such functions may emerge from the broader role the dorsolateral prefrontal cortex has in spatial working memory. These results argue against the dorsolateral prefrontal cortex constituting part of the neuronal substrate responsible for general aspects of implicit or explicit sequence learning.

Introduction

Skill acquisition involves a distributed network of neuronal structures; however, the part each area plays within the overall process remains obscure. Into this knowledge vacuum have swept a number of theories each describing the function of parts of this network. This is perhaps particularly true of the dorsolateral prefrontal cortex, which has been implicated in procedural sequence learning by functional imaging studies (Jenkins *et al.*, 1994; Hazeltine *et al.*, 1997). Consistent with this area providing an essential function during skill acquisition are the deficits observed in those suffering from frontal lobe damage (Gomez-Beldarrain *et al.*, 1999). Moreover, repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex also prevents skill acquisition when the hand contralateral to the stimulated cortex is performing the sequence (Pascual-Leone *et al.*, 1996).

Two broad types of theories have been proposed about the role played by the dorsolateral prefrontal cortex during sequential learning: those which suggest a general function as opposed to those which posit a role specific to particular aspects of a task. A general feature of all types of skill learning is the requirement for attention until the task becomes automatic, hence it has been suggested that the dorsolateral prefrontal cortex role in learning is related to this need for attention (Shallice, 1982; Passingham, 1998). In a related argument, the dorsolateral prefrontal cortex may have an executive role in the organization of skill learning and hence be necessary for all types of action learning (Schwartz *et al.*, 1991). Alternatively, the function of the dorsolateral prefrontal cortex may be more specifically related to aspects of the task, for example acting as a conduit or organizer for particular modalities of sensory information (Goldman-Rakic, 1998).

Sequence learning has been widely investigated using the serial response time task (SRTT). Traditionally, the position of the stimulus has been used as a cue to drive sequence learning; however, it is also possible to use the color of the stimulus as a cue or to use both of these cues simultaneously to drive motor learning. Clearly, sequence learning is the common component of these three tasks; however, the cues responsible for driving this process do vary. Hence, across these three tasks, it becomes possible to dissociate the general aspects of sequence learning from the more specific aspects related to the cues guiding skill acquisition. Here we set out to explore the role played by the dorsolateral prefrontal cortex in sequence learning across these three tasks using rTMS to disrupt the normal functioning of this area. This technique is being increasingly used as a method to establish a causal relationship between an area of cortex and a type of behavior (Pascual-Leone *et al.*, 1999). In this case, disrupting function may impair learning across all three tasks, consistent with the dorsolateral prefrontal cortex having a general role to play in skill acquisition. Alternatively, its effects may specifically prevent learning in only one of the tasks, in accord with a more specific role being played by the part of the dorsolateral prefrontal cortex targeted by rTMS during sequence learning.

Methods

Subjects

We studied six neurologically normal subjects, all of who met the additional safety criteria for rTMS (Pascual-Leone *et al.*, 1993; Wasserman, 1998). All were naïve to the experiment but had previously experienced rTMS. All the subjects were under 30 years of age, four of them were male and only one was left-handed. Critically, none had a personal or a family history of seizures, a history of neurosurgical procedures, closed head trauma or skull lesions. All gave informed consent to the study that had been approved by the institutional review board. rTMS was applied under an Investigational Device Exemption from the Food and Drug Administration (FDA).

SRTT

We used three versions of the SRTT. In the position task, a circular target could appear at any one of four possible positions within an equally spaced horizontal array. Each of the four possible positions corresponded to one of four buttons on a response pad, upon which the subjects' fingers rested. For the color task, the targets all appeared in the center of the screen, with each color corresponding to a unique button on the response pad. The buttons were arranged as a horizontal array; moving from right to left they corresponded to the colors red, blue, yellow and green. Finally, the combined task used a combination of these cues; hence the targets not only occupied different positions, but were also of different colors. Both the color and position stimuli were concordant across and within tasks. In the combined task the cues corresponded to the same response buttons as used in the other two tasks (Fig. 1).

When a target appeared, subjects were instructed to respond by pressing the appropriate button on the pad as quickly and as accurately as possible. Upon giving the correct response the stimulus on the screen disappeared and there was then an interval of 400 ms before the next target appeared. If an incorrect response button was pressed, the stimulus remained until the correct button was selected.

To reduce skill transfer, each task and site had an exclusive 10-item sequence: position task following frontal (4–1–2–4–3–2–1–4–1–3) and parietal (3–1–4–1–2–3–4–2–1–4) stimulation; color task following frontal (3–2–4–3–1–4–2–3–4–1) and parietal (1–4–3–2–4–1–3–4–2–3) stimulation; and, finally, the combined task following frontal (2–1–3–2–4–3–1–3–2–4) and parietal (4–2–3–1–3–4–2–3–1–2) stimulation. Each of the numbers in brackets corresponds to a button on the response pad. All of these sequences were ambiguous with similar complexity. The effect of order of these tasks was counter-balanced across the subjects. Thus each subject performed the tasks in a unique order, because there were six possible task combinations and six subjects. Across each site and task the 10-item sequence was repeated 35 times (350 trials), sandwiched between two blocks of 100 random trials. At the end of the experiment subjects were questioned as to whether or not they had noticed a repeating sequence. Previous experiments have suggested that recognizing a sequence after this relatively limited exposure would be unlikely, implicit sequence learning even following a greater exposure has been a robust finding (Robertson and Pascual-Leone, 2001).

Transcranial Magnetic Stimulation (TMS)

First, we determined each subject's motor threshold and optimal scalp position for a given target muscle following current recommendations by the International Federation for Clinical Neurophysiology (Wassermann, 1998). The site of TMS stimulation and the intensity used to disrupt underlying cortical activity were based on these initial measurements. Seated in a comfortable reclining chair, a subject was able to relax their whole body. Throughout this stage both arms and hands were supported and relaxed. A tightly fitting lycra swimming cap was placed on their head to mark the site of stimulation. Two disposable self-adhesive electrodes (Dantec, Skovlunde, Denmark) were placed on the belly and tendon of the abductor pollicis brevis (APB) muscle of their preferred hand. A ground electrode with a diameter of 30 mm was placed on the wrist. All of these sites were thoroughly abraded and cleaned before the electrodes were attached. Motor-evoked potentials (MEPs) were recorded using a Dantec Counterpoint electromyograph with a band pass filter of 20–1000 Hz.

TMS was delivered with a commercially available 70 mm figure-of-eight coil and a Magstim Super Rapid Transcranial Magnetic Stimulator (Magstim Company, Dyfed, UK). Motor threshold (MT) was defined as the minimal intensity of stimulation capable of inducing MEPs of >50 mV peak-to-peak amplitude in at least 6 out of 10 trials. Stimulation was started at suprathreshold intensity (generally 90% of the stimulator output) and decreased in steps of 2% of the stimulator output. The threshold determination was made during complete muscle relaxation that was monitored on an electromyogram (EMG) for 50 ms prior to the application of the TMS.

We defined the optimal scalp position as the site from which TMS elicited MEPs of maximal amplitude in the contralateral APB muscle. This position was taken to represent the location of the primary motor cortex (Wasserman *et al.*, 1996). The coil was positioned tangentially to the scalp

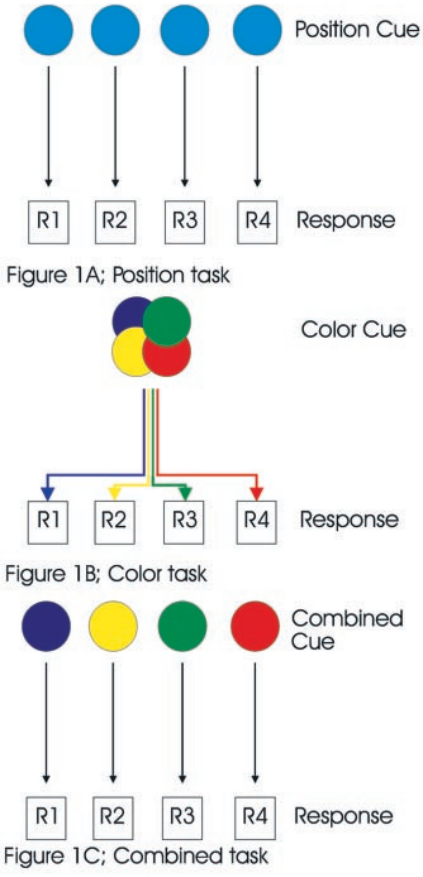

Figure 1. A schematic diagram showing the relationship between stimulus cue and response across the three sequence learning tasks. Only a single stimulus appeared at any time in each task, upon selecting the correct response the stimulus disappeared to be replaced 400 ms later by a further cue. (*A*) Position task — a single blue circle appeared at one of four possible positions within a horizontal array. The position of the stimulus dictated the correct response on a response box. There was a simple spatial correspondence between the stimulus and the response. (*B*) Color task — each stimulus appeared as a single, circular cue at the center of the monitor screen. Each cue had a distinct color, which dictated the correct response. Hence, not only was a sequence acquired in this task, but also an arbitrary visuomotor association between color and response. This additional learning may explain the consistently raised response times observed in this task. (*C*) Combined task — both stimulus position and color were used to cue the correct response. The simple mapping between cue position and response was identical to that used in the position task. Mutually consistent with this relationship was the visuomotor association linking the color and response, which was identical to that used in the color task. Hence this task supplied redundant sensory cues, which were probably responsible for the augmented learning observed in a recent psychophysics study and following rTMS at the control site (parietal cortex).

with the handle of the coil 135° from the midsagittal axis of the subject's head and the coil pointing anterior. This orientation was chosen based on the finding that the lowest MT is achieved when the induced electric current in the brain is flowing approximately perpendicular to the line of the central sulcus (Brasil-Neto *et al.*, 1992; Mills *et al.*, 1992).

Sites of TMS

For stimulation of the dorsolateral prefrontal cortex, the center of the stimulation coil was placed on the lateral convexity 50 mm rostral to the optimal scalp position for induction of MEPs of maximal amplitude in the contralateral APB muscle. This position was based upon coordinates from a stereotactic atlas assuming that the optimal scalp position corresponded to the site of the motor cortex. This definition corresponds to that used in a previous study exploring the role of the dorsolateral prefrontal cortex in procedural sequence learning with rTMS (Pascual-Leone *et al.*, 1996). This placement of the TMS coil is expected to lead to stimulation of the

mid-dorsolateral prefrontal cortex, on the posterior third of the mid frontal gyrus. We also stimulated the parietal cortex. The center of the coil was placed at either P3 or P4 (the site was always contralateral to the hand used to perform the task) according to the international 10–20 system positions for closely spaced electrodes (Morris *et al.*, 1986). Using circular stereotactic markers (Neuroscan Corporation) at these TMS sites a magnetic resonance image (MRI) was taken from two subjects to confirm the anatomical site of stimulation. A T1-weighted image was produced with a Siemens 1.5 T Vision Magnetom MR system using a standard quadrate head coil (Siemens Corporation; MPRAGE sequence, 1 mm isotropic voxels). A total of 160 slices were taken in both sagittal and coronal orientations. The site of stimulation was assumed to be the brain cortical region directly under the projection of the center of the stereotactic marker. For identification of this cortical site a line was projected from the center of the stereotactic capsule into the brain perpendicular to the tangent of the scalp at the location of the marker (Fig. 4).

Paradigm

The experiment was divided into three blocks so that each task was performed once following rTMS. Each block started with 1 Hz rTMS for 5 min (300 pulses) at 15% above the subject's MT, contralateral to the preferred hand to temporarily disrupt cortical processing (Chen *et al.*, 1997; Kossyln *et al.*, 1999). Immediately following this, one of the three versions of the SRTT was performed. Instructions on how to perform the task were given before the start of rTMS. Having completed the task there followed a 10 min rest period, to allow any residual effect of the rTMS to dissipate. This stimulation protocol was based upon an observed 7–10 min reduction in cortico-spinal excitability following a period of rTMS equivalent to that used in our study (Chen *et al.*, 1997). As each task took at least 5 min to complete, a further 10 min rest period led to the rTMS no longer having a significant influence upon cortical excitability. Hence, it took at least 45 min to complete all three tasks, with an additional 15 min to find the motor threshold. To minimize the potential effects of fatigue on this protocol each site was investigated during separate sessions. At least a couple of days separated each session, reducing any possible effect of the order of stimulation.

Using this same protocol, disruptive effects on visual perception and visual imagery were observed following rTMS of the visual cortex (Kossyln *et al.*, 1999). This design has a substantial advantage over the form of rTMS applied in a previous study of the role of the dorsolateral prefrontal cortex in sequence learning (Pascual-Leone *et al.*, 1996). In our current design subjects performed the SRTT task without distraction from concurrent rTMS. This minimizes non-specific disruptive effects of rTMS and hence significant results are most likely true consequences of physiologic effects of the stimulation (Pascual-Leone *et al.*, 1999; Kossyln *et al.*, 1999). Despite the considerable advantages of this paradigm we still controlled for any non-specific effects of rTMS by comparing across sites and tasks. This approach has acquired a consensus across a wide range of contemporary TMS studies (Jahanshahi and Rothwell, 2000).

Experimental Design

For all the tasks we measured response time: the interval between the appearance of the visual stimulus (target) on the computer screen and the time of depression of the correct response key. Each target was a circle (diameter 35 mm) and its presentation was controlled by a computer (Pentium PC) using software designed to record response times (Superlab Pro). Subject responses were measured using a four-button response box (Cedrus RB-410) connected to the serial port of the computer. During the experiment subjects were instructed to rest the index, middle, ring and little fingers of the hand on the appropriate response keys, while they viewed the computer monitor from ~600 mm.

Data Analysis

Response time was defined as the interval between the appearance of the visual cue on the screen and the time of the correct response. Thus, selecting an incorrect response, a so-called error, would be reflected in an increased response time. Allowing the frequency and extent of errors to influence response times seems appropriate because without such an interaction it would be possible for response times to be sent artificially low by a series of erroneous responses. For each site of stimulation the subject, task and their interaction effect on learning were explored using an analysis of variance (ANOVA). So, a difference in the amount of learning across the three tasks was statistically tested. In addition, with each subject randomly assigned to a unique order of tasks, any effect of subject reflected a significant influence of task order. An ANOVA was also used to compare the extent of learning across the two sites but within the same task. Thus a functional dissociation between the role of the dorsolateral prefrontal and parietal cortices in a sequence learning task was tested statistically.

Learning within each task was defined as a significant increase in response times during the final 100 random trials compared to the earlier 10-item sequence trials. Any effect upon movement would be present in both the sequential and the random trials. So, if exclusively motor performance were affected, then a comparison between sequential and random trials would fail to show a significant difference. Hence, by defining procedural learning as a significant increase in response time during the random trials the potential confound between motor performance and learning was removed. This empirical definition of skill acquisition, the so-called 'after-effect', has been used in many studies of procedural learning (Martin *et al.*, 1996; Gomez-Beldarrain *et al.*, 1999; Robertson and Miall, 1999). We have used this definition in a previous study and were able to observe sequence learning across all three tasks (Robertson and Pascual-Leone, 2001). Within each task a *t*-test was used to compare the response times during the random and sequential trials and so establish the presence of procedural learning. A significant rise in response times during the random trials was interpreted as evidence of procedural learning. To reduce the possibility of artefacts and so increase confidence in the validity of the contrasts, we did not limit the analysis to sampled data from the learning curves or averaged values from blocks of trials. Instead, a complete set of response times was used to make the comparisons across both task and stimulation site. An ANOVA was used to compare the random trial response times across sites to ensure that changes in these response times were not responsible for differences in sequence learning. For all of these statistical tests a significance level of $P < 0.05$ was adopted.

Results

Dorsolateral Prefrontal Cortex

Initially, the effect upon sequence learning of both the subject and the task performed was explored. While the sequence learning task had a significant effect upon the extent of learning (ANOVA, $F = 12.9$, $P = 0.002$), there was no significant effect of subject (ANOVA, $F = 1.2$, $P = 0.367$). Hence, we went onto examine the influence of task upon sequence learning.

During the position task, with prior stimulation of the dorsolateral prefrontal cortex there was no significant difference between the response times of the repeating sequence and the subsequent random trails (*t*-test, $P = 0.19$, Fig. 2, Table 1). This implies that procedural sequence learning, guided by position, was prevented by prior rTMS. However, a significant difference between the response times of the repeating sequence and the subsequent random trials was present when procedural learning was guided by either color alone (*t*-test, $P = 0.04$, Fig. 2, Table 1) or by color and position (*t*-test, $P = 0.01$, Fig. 2, Table 1). So despite rTMS, procedural learning was observed in both the color and the combined tasks. Moreover, the extent of learning in both of these tasks was similar (ANOVA, $F = 1.8$, $P = 0.2$). Regardless of whether the trial was part of a repeating sequence or random, the color task showed significantly higher response times than either of the other tasks (position, *t*-test, $P = 0.01$; combined, *t*-test, $P = 0.01$). Despite these differences, none of the subjects became aware of sequence within any of the tasks.

Parietal Cortex

The sequence learning task had a highly significant effect upon

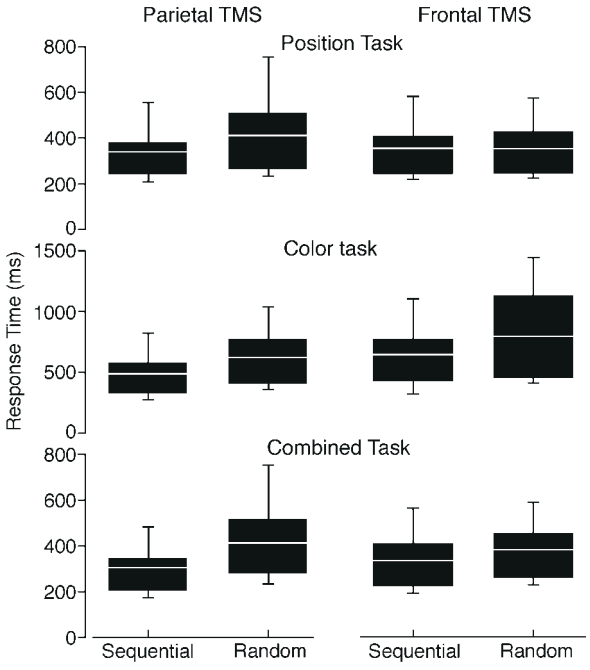

Figure 2. Sequence learning is prevented exclusively in the position task following frontal stimulation. Moreover, while the combined task shows significantly greater learning than either the color or the position task at the parietal site, this enhancement is lost following frontal stimulation, demonstrated by the color and combined tasks showing equivalent learning. This implies that rTMS at the frontal site is able specifically to disrupt the use of spatial information to guide sequence learning. Thus, learning is prevented in the position task and the combined task no longer shows the enhancement of learning, consistent with only the color cue being available to guide learning. Procedural sequence learning was defined as a significant increase in response times during the performance in the random trials following earlier performance of a repeating 10-item sequence. This comparison is shown for each task following stimulation at both frontal and parietal sites, for a representative subject. For each task across the two sites, the box plot on the left shows the response times during the repeating 10-item sequence, while the box on the right shows this for the subsequent random trials. Within each box, the central white line shows the mean of the distribution, with the box enclosing the 25–75th percentiles and the whiskers showing the 10th and 90th percentiles.

Table 1
Response times for all subjects in all three tasks following rTMS at both the dorsolateral prefrontal and parietal cortices

	Tasks	Response times (ms)	
		Sequential	Random
Dorsolateral prefrontal cortex	position	375 ± 54	382 ± 55
	color	534 ± 67	712 ± 66
	combined	291 ± 70	379 ± 66
Parietal cortex	position	355 ± 68	452 ± 62
	color	593 ± 69	721 ± 54
	combined	280 ± 59	422 ± 77

A significant rise in response time during the random trials, the so-called 'after effect', was used as evidence of skill acquisition. Both the after effect and the absolute value of the response times following rTMS at the parietal cortex are similar to those observed previously without stimulation (Robertson and Pascual-Leone, 2001). Learning is present across all three tasks and is significantly greater in the combined task. However, following frontal stimulation learning is prevented in the position task and the augmented learning within the combined task is no longer observed. Nonetheless, learning within the color task is unaffected; hence rTMS of the dorsolateral prefrontal cortex specifically prevents spatial cues guiding sequence acquisition.

the extent of learning (ANOVA, $F = 58.7$, $P < 0.0001$), but there was no significant effect of subject (ANOVA, $F = 1.8$, $P = 0.216$). Hence, again we were able to examine the effect of the task upon sequence learning.

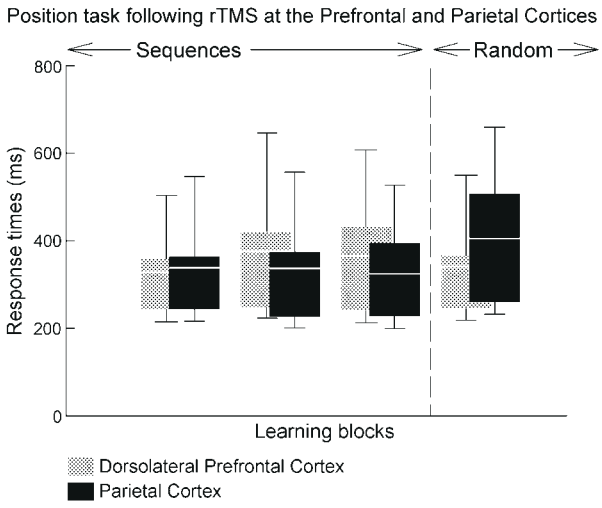

Figure 3. The progressive changes in response times during the position task following rTMS at both the dorsolateral prefrontal cortex and the parietal cortex. The response times following frontal stimulation show very little change across the sequence trials and, even more importantly, there is no significant rise in response time during the random trials. This so-called 'after effect' is a method commonly used to demonstrate that an improvement in performance is specifically due to learning. This feature is clearly present following rTMS at the parietal cortex, indicating the successful acquisition of the sequence. Moreover, response times fell steadily during the sequence trials, consistent with sequence learning being able to occur despite the earlier rTMS at the parietal site. The data shown are from a single subject, not shown in Figure 2, with the first 50 sequential trials removed and every following 100 trials represented as a single box plot. The box plot conventions are the same as those used in Figure 2.

Despite preceding rTMS of the parietal cortex, there was still a highly significant difference between the response times of the repeating sequence and the random trials during the position task (*t*-test, $P = 0.006$). This is in contrast to the absence of sequence learning in the position task following stimulation at the dorsolateral prefrontal cortex (Fig. 2, Table 1).

The functional dissociation provided by rTMS at these cortical sites, dorsolateral prefrontal and parietal cortex, was consistently maintained throughout exposure to the position task (Fig. 3). Examining the time course of responses following prefrontal stimulation, there was no evidence of the late emergence of learning; however, steadily decreasing response times were observed following parietal stimulation (Fig. 3). Hence, the functional dissociation provided by rTMS between these cortical sites was consistently maintained throughout exposure to the position task.

Similarly, in the color task there was a significant difference between the response times of the repeating sequence and the subsequent random trials (*t*-test, $P < 0.0001$, Fig. 2, Table 1). Procedural sequence learning was also observed in the combined task (*t*-test, $P < 0.0001$, Fig. 2, Table 1). There was no difference in the extent of the skill acquired in the position and color tasks (ANOVA, $F = 2.64$, $P = 0.179$); however, there was significantly greater learning in the combined task (ANOVA, $F = 10.31$, $P = 0.03$). This implies, as observed in a previous study (Robertson and Pascual-Leone, 2001), that there was greater learning in the combined task than in either of the other two tasks. Once again, the color task showed significantly higher response times than either of the other tasks (position, *t*-test, $P = 0.001$; combined, *t*-test, $P = 0.001$). Although parietal cortex stimulation gave a different pattern of learning across the three tasks, the subjects never reported the presence of a sequence. This same lack of awareness was also found following rTMS at

the dorsolateral prefrontal cortex. Hence, as would be expected for such limited exposure to a 10-item sequence, the learning never became explicit. In addition, response times also remained greater than a visual reaction time and so failed to show evidence of being preparatory, a feature of explicit learning (Willingham *et al.*, 1989). Consequently, the different pattern of skill acquisition associated with rTMS at these cortical sites cannot be ascribed to differences in the awareness of a sequence during exposure to each of the tasks.

In summary, there was significantly more learning in the position task following parietal rTMS than after stimulation to the dorsolateral prefrontal cortex (ANOVA, $F = 56.4$, $P = 0.002$). Site of rTMS (prefrontal versus parietal) did not affect learning in the color task (ANOVA, $F = 1.542$, $P = 0.282$). Critically, across all three tasks, there was no significant effect of site upon the responses times during the final block of random trials (ANOVA, $F = 2.27$, $P = 0.103$). Thus, the response times achieved during the sequential trials were being compared against a common baseline.

Anatomical MRI Confirmation

In two subjects, vitamin E capsules were used to visualize the sites of rTMS on an anatomical brain MRI scan. For the frontal site, the study in both subjects showed that the center of the figure-of-eight coil had been placed directly above the middle frontal gyrus, contralateral to the preferred hand (Fig. 4). Thus, stimulation at this site would almost certainly have disrupted the function of the dorsolateral prefrontal cortex (area 46) contralateral to the hand used in task performance. The 10–20 system for electrode placement was used to determine the site of parietal stimulation. The MRIs in both subjects studied confirmed that the coil had been placed above the inferior parietal gyrus and so stimulation is likely to have affected area 40 and surrounding areas.

Discussion

Here we confirm earlier results by showing that sequence learning can be prevented by rTMS at the dorsolateral prefrontal cortex (Pascual-Leone *et al.*, 1996). This was achieved despite rTMS preceding the learning task, showing that cortical disruption can outlast the period of stimulation. Our observations also demonstrate that the critical contribution of the dorsolateral prefrontal cortex is robustly related to the sensory cue driving sequence learning. These findings offer some fresh insight into the contribution of the dorsolateral prefrontal cortex to sequence learning.

When a spatial cue alone was used to guide learning, disruption of dorsolateral prefrontal cortex function prevented skill acquisition. In contrast, learning guided exclusively by a color cue was unaffected by prior stimulation at the dorsolateral prefrontal. Finally, the relationship between the color and the combined task was disrupted: both showed the same degree of learning following prefrontal stimulation. Previously, sequence learning was found to be enhanced when both color and position acted as cues (Robertson and Pascual-Leone, 2001). Hence, the results in the combined task are consistent with only a single cue being available to drive learning following rTMS of the dorsolateral prefrontal cortex. As in the position task, disrupting cortical function probably prevents spatial stimuli acting as effective cues. None the less, learning is still able to occur because the color cue in the combined task compensates for this deficit. These results suggest that the critical contribution of the dorsolateral prefrontal cortex to sequence

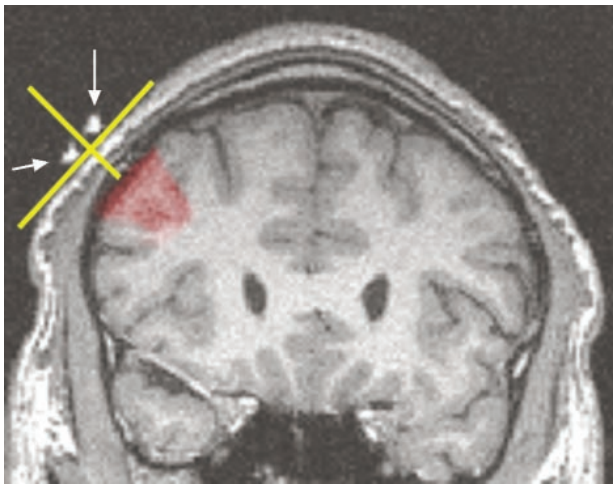

Figure 4. Coronal anatomical MRI of a representative subject, showing the site at which rTMS was delivered to target the dorsolateral prefrontal cortex. On the left-hand side of the image, above the skull can be seen the stereotactic marker used (arrows). In this coronal cut the marker appears in two parts due to its ring shape. The marker was applied to the subject's scalp position that was directly under the center of the coil during stimulation. The tangent to the scalp at this location and a perpendicular line to it are drawn in. As can be seen, the center of the stereotactic marker projects directly above the middle frontal gyrus and rostrally enough to be targeting the dorsolateral prefrontal cortex.

acquisition is related to the processing of available spatial cues for the guidance of learning.

This learning impairment is unlikely to be a non-specific effect of rTMS. First, the effects of prefrontal stimulation are specific for a given sensory cue and our results are consistent with previous studies demonstrating impairment in sequence learning when guided by position, following damage or disruption to this area (Pascual-Leone *et al.*, 1996; Gomez-Beldarrain *et al.*, 1999). Second, our findings cannot easily be explained on the basis of interference with anatomical structures in the proximity of the targeted prefrontal cortex. The frontal eye fields are perhaps the only other frontal structure that has been implicated in processing specifically spatial cues and which may play a subsidiary role in sequence learning. However, it seems unlikely that rTMS could have directly influenced a site so distant from the targeted dorsolateral prefrontal cortex. The frontal eye fields are at least several centimeters from the dorsolateral prefrontal cortex, the target of rTMS in this experiment. This compares with the estimated 5–10 mm over which rTMS is thought to have an influence on cortical sites (Brasil-Neto *et al.*, 1992). Indirect stimulation of the frontal eye field may have induced eye movements, but although looked for these were never observed. Nor were any hand or arm movements induced by rTMS, which suggests that stimulation did not extend into the motor cortex. Moreover, the anatomical MRI scans confirmed that the center of the coil was placed over the dorsolateral prefrontal cortex and, hence, rTMS is likely to have disrupted its function (Brasil-Neto *et al.*, 1992; Wilson *et al.*, 1993). Finally, stimulation of the parietal area was unable to prevent learning regardless of the sensory cue guiding skill acquisition, a finding consistent with earlier work (Pascual-Leone *et al.*, 1996). A comparison of the effects of rTMS across sites is a useful approach to ensure that the non-specific effects of TMS do not allow misleading interpretations of cortical function.

It is important to emphasize that our results are not evidence for a lack of involvement of the parietal cortex in sequence

learning, merely that its possible role was obscured in our behavioral paradigm. A recent functional imaging study might be interpreted to support such a non-critical role of this parietal cortical area by implicating it in the representation of a sequence at an abstract level independent from the particular effectors used to perform the task (Grafton *et al.*, 1998). However, we found that stimulation of the parietal cortex results in a learning enhancement within the combined task. This unexpected result might suggest that disruption of parietal cortex by rTMS can lead to a functional disinhibition of the prefrontal cortex resulting in a paradoxical behavioral gain. However, a simpler explanation would be to postulate that rTMS to the parietal cortex has no effect on sequence learning and that the observed learning enhancement is the expected consequence of exposure to the combined task. The parietal cortex might have a critical role in transfer rather than in the acquisition of sequence learning. Nevertheless, further experiments are needed to resolve this issue.

'Off-line rTMS'

These results also expand upon previous findings by demonstrating that rTMS disrupts sequence learning even beyond the application of the stimulation itself. This has the substantial advantage of minimizing the influence of non-specific concurrent effects of rTMS on behavior. Consequently, the pattern of observed deficits is likely to result exclusively from the disruption of function within a targeted cortical area. These 'off-line rTMS effects' open up new avenues of research into cortical function, allowing a more effective integration of rTMS with functional imaging and the development of behavioral paradigms which would be unsuitable or interact with concurrent rTMS.

Cue-dependent Contribution to Sequence Learning

Numerous studies have demonstrated both the involvement of the dorsolateral prefrontal cortex as well as its critical role in sequence learning (Jenkins *et al.*, 1994; Pascual-Leone *et al.*, 1996; Hazeltine *et al.*, 1997; Gomez-Beldarrain *et al.*, 1999). However, how it performs this role has remained uncertain. Novel actions are frequently performed with difficulty and generally lack skill. Consequently, until a novel action has become well learnt, it requires attention. This very general quality of skill learning is thought to depend critically upon the dorsolateral prefrontal cortex (Passingham, 1998). Alternatively, the dorsolateral prefrontal cortex may have a more direct but nevertheless general role to play in sequence learning. Perhaps this area acts as the neuronal substrate for processing many distinct types of temporal information and may thus have a pivotal role to play in sequence learning (Fuster, 1990, 1992). These diverse theories have a basic feature in common: disruption of the dorsolateral prefrontal cortex should lead to impairment in all types of sequence learning, regardless of the modality guiding the acquisition of skill. However, this is inconsistent with our results: sequence learning guided by position was prevented following rTMS and the expected augmentation of learning within the combined task was not observed, consistent with only a color cue being available to guide learning. Consequently, the dorsolateral prefrontal cortex appears to have a specific role to play in sequence learning, responsible perhaps for the temporal organization and learning of information based exclusively upon spatial cues.

Learning Circuits

A composite role for the dorsolateral prefrontal cortex in both the organization and acquisition of spatial sequences is consistent with our current observations. However, this implies that exclusively the dorsolateral prefrontal cortex is critical to sequence learning when guided by a spatial cue. Yet other brain areas, such as the cerebellum, are critical to sequence learning when guided by spatial or non-spatial cues (Molanari *et al.*, 1997; Gomez-Beldarrain *et al.*, 1999). Consequently, the dorsolateral prefrontal cortex is probably a vital component within a neural circuit of cortical and sub-cortical areas, each making a critical but distinct contribution to sequence learning. Potentially, this neural circuit may reflect our observations of the dorsolateral prefrontal cortex by also being dedicated exclusively to sequence learning guided by a spatial cue. Thus there would be an array of parallel circuits each dedicated to a specific guiding cue, a perspective with some anatomical merit (Middleton and Strick, 2000).

However, observations from this and previous studies have demonstrated an augmentation in sequence learning when guided by congruent spatial and non-spatial cues. Hence, a functional interaction occurs amongst the cues, making it unlikely that an array of dedicated parallel circuits will be sufficient as a neural organizing principle for sequence learning. Further doubt is cast upon this principle by the observed asymmetrical transfer between the color and position tasks: skill can be transferred from the color to the position task but not vice versa (Robertson and Pascual-Leone, 2001). This learning property is unlikely to emerge from an array of cue-dependent circuits. Instead we have suggested that sequence acquisition is achieved by an interaction and co-operation between cue-dependent and independent circuits (Robertson and Pascual-Leone, 2001). Thus, we envisage the prefrontal cortex as a cue-dependent component within this wider neural circuit.

Processing within the Dorsolateral Prefrontal Cortex

Irrespective of the organizing principles underlying the neural circuit for sequence learning, our observations offer some insight into the contribution made by the dorsolateral prefrontal cortex to this circuit.

The observed modality-specific impairment in sequence learning following rTMS at the dorsolateral prefrontal cortex is inconsistent with it making a general contribution to all types of sequence learning. Consequently, supplying a critical contribution to the sequencing of behaviors, the temporal organization of events, the attention required to perform novel tasks or other executive supramodal functions seems an unlikely role for the dorsolateral prefrontal cortex during sequence learning (Shallice, 1982; Fuster, 1990, 1992, 2000; Schwartz *et al.*, 1991; Passingham, 1998).

Based upon behavioral, anatomical and electrophysiological evidence, it has been argued that the dorsolateral prefrontal cortex acts as a temporary store of modality-specific information: receiving and processing sensory information related to spatial stimuli and retaining this information over the short term so that a task may be performed (Levy and Goldman-Rakic, 2000). Another related theory views the prefrontal cortex as a mosaic of supramodal and modality-specific areas (Petrides, 2000a). However, at least for the dorsolateral prefrontal cortex, both these perspectives are mutually consistent by asserting that this area of cortex makes a critical contribution to spatial working memory. The precise nature of this contribution is uncertain; a recent study suggested that this area of prefrontal cortex was

responsible for the manipulation of spatial cues rather than their retention (Petrides, 2000b). In our paradigm it may be artificial to draw a distinction between these processes because an ability both to retain and manipulate the relative spatial location of stimuli is likely to be critical to the acquisition of a sequence. Hence, we suggest that the critical contribution of the dorsolateral prefrontal cortex to sequence learning is to retain, manipulate or transform spatial cues in the relatively short term before this information is used by other cortical and subcortical areas. This contribution may emerge from the broader role which the dorsolateral prefrontal cortex has in spatial working memory. The ventromedial prefrontal cortex may well act in a similar capacity for color information (Passingham, 1993; Hazeltine *et al.*, 1997).

Cue-dependent Brain Areas and the Representation of Action

Those anatomical structures recruited during sequence learning seem at least partly determined by the type of sensory information available; after all, acquiring a sequence within the color task was not critically dependent on the dorsolateral prefrontal cortex. However, eventually all motor tasks must come to be represented by the motor cortex, regardless of how an action was initially acquired. Nevertheless, it seems likely that the nature of this representation will reflect how a skill was acquired. Recent functional imaging studies have demonstrated that an identical movement sequence learnt with color as opposed to position as a cue is associated with a distinct pattern of activation across the primary motor cortex (Hazeltine *et al.*, 1997). Thus, the history of how a movement was learnt has an important role to play in determining not only the anatomical structures critical for learning, but also the nature of the final representation of an action. Certainly, these observations suggest that a representation of action can not exclusively be reduced to features of the movement itself or to particular aspects of a task, even in the primary motor cortex. Hence, the difficulty patients with frontal lobe damage have in learning a novel sequence may result from an inability to use sensory information to drive skill rather than poverty in the control of movement (Gomez-Beldarrain *et al.*, 1999). Dysfunction of the frontal lobes, particularly for the short-term retention of spatial information, is also a feature of Parkinson's disease (Robbins, 2000). Thus our observations may also provide an explanation for the impairment of sequence learning which has been observed in patients with advanced Parkinson's disease (Pascual-Leone *et al.*, 1993).

Notes

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