Section 9: Investigating the functional significance of ipsilateral motor cortical activation: Temporary interference by TMS can slow ipsilateral motor responses

Previous sections in this thesis have identified contexts in which movement-related FMRI activation is detected in the hemisphere ipsilateral to the hand being moved. In Section 4 it was shown that movement of an affected hand after recovery from stroke is associated with a more bilateral pattern of activation than movement of an unaffected hand. Section 5 demonstrated that more bilateral patterns of movement-related activation are seen in normal subjects performing complex hand movements. These experiments have demonstrated tasks and subject groups in which movement is associated with ipsilateral activation but they are unable to demonstrate the functional relevance of ipsilateral activation. In this section transcranial magnetic stimulation (TMS) is used to transiently interfere with processing in ipsilateral motor cortical areas. This approach is used to test the involvement of the ipsilateral hemisphere in simple and complex movements in normal subjects and patients after stroke.

9.1 The effects of movement complexity and site for TMS-induced disruption of motor responses in normal subjects

In normal subjects, motor cortical areas in the ipsilateral hemisphere can be involved in hand movements, particularly if the movements are complex (see Section 5). The role of the primary and premotor cortices differs in the control of ipsilateral hand movements. For example, one PET study showed that the premotor cortex is particularly involved in ipsilateral movements that require movement selection (Schluter *et al.* 2001) . This effect is lateralised, with the left premotor cortex dominant for the selection of ipsilateral hand movements (Schluter *et al.* 2001). The functional relevance of ipsilateral activation associated with movements can be tested by using TMS as a 'virtual lesion' technique (Section 3.2.2).

9.1.1 Introduction and rationale

The functional relevance of ipsilateral motor cortical activation in normal subjects is unclear. Results from studies that have used TMS to transiently disrupt motor cortical processing may help address this issue.

Schluter et al (1998) used single pulse TMS over contralateral and ipsilateral motor and premotor areas during performance of two different visually-cued motor tasks. In one task (choice reaction time) subjects responded to small circles or large rectangles by pressing a button with their index finger and to large circles or small rectangles by pressing a button with their middle finger. In a second task (simple reaction time) subjects responded to all visual cues by pressing a button with their index finger. Effects of TMS on reaction times (RTs) were influenced by the site and time of the TMS pulse, and by the task being performed. During the choice RT task, responses were delayed by early (100-140ms post cue) TMS of contralateral premotor cortex (PMC) or late (300-340ms) TMS of contralateral primary motor cortex (MC). During the simple RT task, responses were delayed by late contralateral MC stimulation but not by early contralateral PMC stimulation. *Ipsilateral* TMS was effective at delaying responses during the choice RT task only when applied early over the (left) premotor cortex. This suggests that the left PMC is involved in movement selection, which is required in the choice RT task and not in the simple RT task. This study therefore demonstrates that the left PMC activation associated with movement selection in the PET study reported above (Schluter *et al.* 2001) is functionally relevant, as interference with this area has behavioural consequences. Furthermore, by using single pulse TMS, the study has demonstrated that the role of left premotor cortex in the selection of ipsilateral movements is limited to a 100-140ms time window after the cue to move (Schluter *et al.* 1998).

Gerloff et al (1998) used repetitive pulse TMS (rTMS) over primary motor cortex during performance of remembered movement sequences of varying complexity. One sequence (simple) involved repetitive movements of the index finger (2-2-2-2-2 etc). A second sequence (scale) involved consecutive pressing of four fingers (5-4-3-2-5-4-3-2 etc). A third sequence (complex) involved four fingers in a non-consecutive order (2-5-4-3-3-5-2-4-5-2-3-4-4-2-5-3).

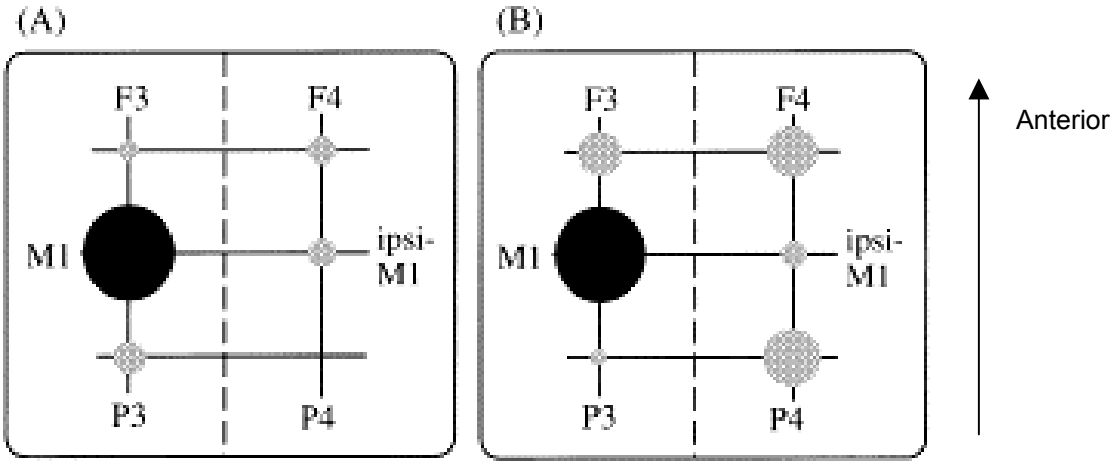

Figure 9.1: Topography of rTMS effects in study by Gerloff et al. The number of errors for each scalp position is illustrated by the diameter of the circles. In both scale (A) and complex (B) sequences the highest number of errors occurred over contralateral M1 (black circles). However, disruption effects were also detected with rTMS of ipsilateral motor sites and the magnitude of these effects was enhanced in the complex conditions. For example the grey circle for the site marked F4 (which may correspond to ipsilateral premotor cortex) is larger in B than A.

Analysis of accuracy and timing errors demonstrated that rTMS of contralateral MC was more effective at disrupting performance of complex sequences. For disruption of simple sequences higher rTMS intensities were required. rTMS was also applied at other scalp locations including ipsilateral MC and an area anterior to this, probably close to premotor cortex. There was a significant effect of scalp position, with stimulation over the contralateral MC site having more effect on performance than other sites. Nevertheless, it is clear from the results that the ipsilateral MC rTMS did have an effect on movement and that this effect was greater for the complex sequences (Figure 9.1), although the significance of these effects was not tested.

The current study aimed to specifically investigate the role of ipsilateral motor and premotor cortices in the performance of simple and complex motor responses. First, FMRI was used to quantify the activation of ipsilateral motor areas during simple and complex finger movements. Second, TMS was used during performance of the same tasks to target ipsilateral motor cortical areas activated in the FMRI experiment.

9.1.2 Methods

Subjects: 16 right handed normal subjects (age 23 to 65, mean 40.7, 8 male, 8 female) participated in accordance with local ethics approval.

FMRI scanning: A 3T Varian/Siemens MRI system was used. Axial echo-planar volumes were acquired (21x6 mm slices, TE=30ms, TR = 3000ms, FOV = 256x256, matrix = 64x64). A T1-weighted anatomical image was also acquired for each subject (IR 3D Turbo Flash, 64x3 or 1.5mm axial slices, TR=30ms, TE=5ms, TI=500ms, flip angle=15°, FOV=256x256, matrix=256x256).

Subjects performed 30 second blocks of visually-cued finger tapping tasks or rest. Finger tapping tasks varied in complexity (index finger tapping (B), sequential finger tapping (1,2,3,4,3,2,1 etc) (C) and random finger tapping (D)). The order of tapping tasks was varied so blocks were performed in an ABCDADCBACBD order where A is rest. The task was performed first with the dominant, then with the non-dominant hand. However, data was only analysed for the hand that each subject used to perform the TMS experiment. Subjects practised the tasks before entering the scanner.

Image analysis: Image analysis was carried out using tools from the FMRIB Source Library (FMRIB, Oxford, UK, www.fmrib.ox.ac.uk/fsl). The following pre-statistics processing was applied: motion correction using MCFLIRT (Jenkinson and Smith 2001); spatial smoothing using a Gaussian kernel of full width half maximum 5.0mm; mean-based intensity normalisation of all volumes by the same factor; nonlinear highpass temporal filtering (Gaussian-weighted least squares straight line fitting, with sigma=90.0s). Statistical analysis was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich *et al.* 2001). Random effects group analyses were carried out and group Z-statistic images were thresholded using Z>3.1, and a cluster significance threshold of p=0.01, corrected for multiple comparisons (Worsley *et al.* 1992; Friston *et al.* 1994; Worsley *et al.* 1992).

The primary aim of this experiment was to explore the relationship between FMRI activation and the effects of TMS. Further analysis was therefore focussed on anatomically-defined volumes of interest (VOIs) corresponding to sites of stimulation in the ipsilateral hemisphere and their homologues in the contralateral hemisphere. The following VOIs were defined for each subject and each hemisphere based on individual subject high-resolution T1-weighted anatomical scans:

1. Sensorimotor cortex: Anterior and posterior banks of central sulcus, posterior half of precentral gyrus and postcentral gyrus from the level of the top of the lateral ventricles to the most dorsal slice of the brain

2. Premotor cortex: Anterior half of precentral gyrus and sulcus from the level of the top of the lateral ventricles to the most dorsal slice of the brain

Registration of VOIs to statistical images was carried out using FLIRT (FMRIBs Linear Image Registration Tool, (Jenkinson and Smith 2001)).

The maximum percent signal change from rest to movement was calculated within each VOI and for each task condition. To investigate effect of task, hemisphere, brain region and hand being moved these values were analysed in repeated measures general linear model (GLM). This was followed up with paired t-tests to identify where differences occurred.

The maximum percent signal change values were used to calculate a laterality index (LI) ([C-I]/[C+I] where C = contralateral and I= ipsilateral max % change). Differences in LI between conditions, brain regions and hemispheres were assessed using paired t-tests.

TMS testing: An initial estimate of the scalp position of the hand area of M1 was marked 4cm lateral and 2cm anterior to the vertex (Cz). The hand area was localised by looking for visible movement of the index finger in response to TMS of points around this scalp mark (Schluter *et al,* 1998). A figure-of-eight stimulation coil was used for

localisation of the motor cortex (each wing 50mm in diameter). The coil was connected to a Magstim Rapid Stimulator (Magstim, Camarthenshire, Wales, UK) with a maximum output of 2 Tesla. The centre of the coil was placed over the estimated M1 and tangential to the scalp, with current flowing in an anterior to posterior direction parallel with the midline. Single TMS pulses starting at 40% of the maximum output of the stimulator and increasing in 5% steps were applied to the estimated M1 until a muscle contraction was observed. The coil was then moved in 5mm steps to points in a grid of approximately 3x3cm centred on the estimated M1. The point where stimulation evoked maximum movement specific to the index finger was located. Stimulation intensity was then reduced to find the threshold above which an observable movement could be reliably detected. Adjacent sites were tested at this threshold to check whether stimulation of alternative points evoked greater movement. If not, then the provisional site was designated the motor 'hotspot'. If stimulation of adjacent sites evoked greater movement then thresholding was performed again and the procedure repeated until a 'hotspot' was found.

Two stimulation sites were marked relative to the motor 'hotspot'. First, a primary sensorimotor (MC) site was marked 1cm posterior to the 'hotspot'. Second, a dorsal premotor site (PMC) was marked 2cm anterior and 1cm medial to the 'hotspot'. Previous studies have shown that stimulation of these two sites leads to dissociable effects on movement execution and selection (Schluter *et al.* 1998).

The 50mm coil used for localisation provided the most specific estimate of the site of the motor 'hotspot'. However, for the remainder of the experiment a slightly larger coil was used (wing diameter 70mm) to increase the likelihood that the crucial region of the dorsal premotor cortex would be targeted by the PMC site stimulation (There is no equivalent to the motor hotspot that provides a functional landmark for the PMC). The motor threshold at the MC was found with the larger coil. As the induced

current would be expected to spread over a greater area with the larger coil the PMC site was stimulated at threshold to confirm that stimulation of that site did not evoke visible movement.

Stimulation was applied at 120% of motor threshold for 14 out of 16 subjects (equivalent to 60-70% of the maximum output of the stimulator). For two subjects with high thresholds stimulation was applied at 115% of motor threshold to minimise discomfort (equivalent to 76-80% of maximum stimulator output).

Half the subjects were assigned to a right hand group and half to a left hand group. They performed two of the finger tapping tasks they had previously performed in the FMRI experiment – the index finger tapping task, and the random finger tapping task. Each task was performed four times, twice with TMS of MC, and twice with TMS of PMC. Tasks were performed in blocks of 60 trials. Movement cues remained on the screen until a response was made and reaction times were recorded. Subjects practised each task without TMS first. On TMS runs stimulation was randomly applied on half of all trials at one of 5 stimulation times: 50, 100, 150, 200 and 250ms after the movement cue. Thus for each stimulation time, each site (MC, PMC) and each condition (simple, complex) subjects performed 10 trials with TMS. For each site and each condition there were 60 control trials with no TMS. Data were analysed for all five time points in the randomly cued task but only the first three time points were analysed in the more quickly performed fixed finger task.

Subjects were seated in front of a computer screen and rested on a chin rest. In between each TMS block they were able to rest if required. After 4 TMS blocks subjects rested while MRI-guided localisation of the TMS coil was performed.

Frameless strereotaxic MRI-guided localisation of TMS sites: Frameless stereotaxy was used to individually localise stimulation sites with respect to anatomical landmarks on each subject's MRI scan (Paus *et al.* 1997). Landmarks visible on the head (tip of

nose, bridge of nose, left and right tragus) were marked on each subject's T1-weighted high-resolution MRI scan. A Polaris infra-red tracking device (Northern Digital, Ontario, USA) was used to detect probes on landmarks and reference points on the subject's head. Brainsight software (Rogue Research, Montreal, Canada) was used to co-register the subject's head with their MRI scan. A probe was attached to the coil that was held over the stimulation sites enabling the localisation of the target sites on the MRI scan.

This approach was used to define the trajectory of stimulation sites on the MRI retrospectively. Pilot studies with the paradigms used here suggested that localising the motor hotspot based on muscle responses provided greater interference effects than targeting stimulation at FMRI activation peaks. In the current study the Brainsight system was used to find the location of physiologically-defined TMS targets.

Analysis of TMS reaction times: Median correct reaction times (RTs) were found for each condition with and without TMS. The percent change in RT from the no TMS baseline was calculated for each timepoint, condition, and stimulation site. The significance of the effect of TMS at each time point was assessed by comparing RT percentage change values to the zero baseline with one-sample t-tests. This was done separately for each stimulation site and each condition and probability values were corrected for the number of time points tested. The effects of task, time of stimulation, site of stimulation and hand being moved were assessed using a repeated measures general linear model (GLM). Comparisons between conditions and sites were made using paired t-tests.

9.1.3 Results

FMRI results Controls activated the expected network of sensori-motor regions during finger movements (Figure 9.2). Activation was detected in the ipsilateral hemisphere for both tasks.

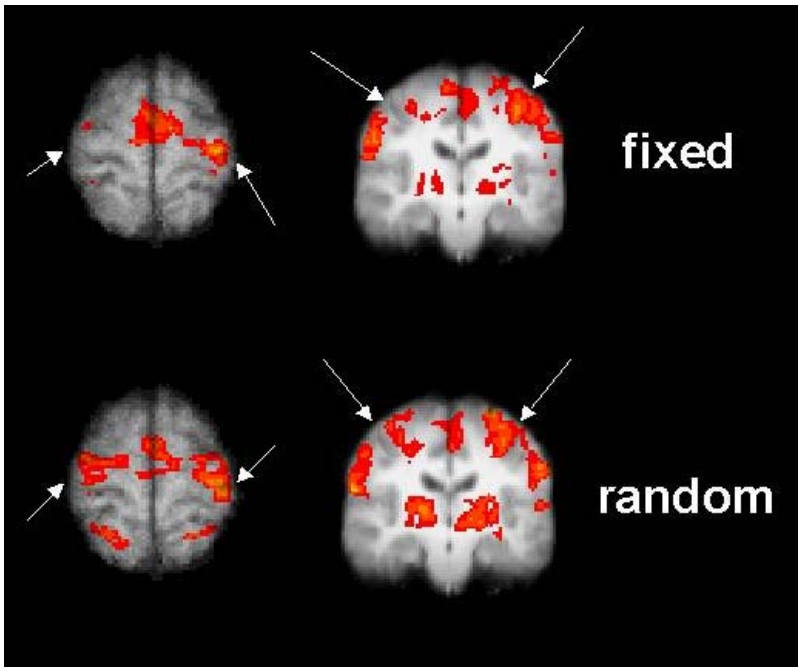

Figure 9.2: In healthy controls, randomly cued finger movements (bottom) produced more overall FMRI activation than fixed index finger movements (top). Activation was also more bilateral for randomly cued movements. Activation from left and right hand groups have been combined by rotating the data for left hand movement about the midline. Images are thresholded at $Z>3.1$ and cluster extent threshold of $p<0.01$. Arrows indicate position of the central sulcus.

The maximum percent signal change within the chosen volumes of interest (VOIs) corresponding to the sites of stimulation and their homologues in the opposite hemisphere (i.e. contralateral and ipsilateral MC and PMC (CMC, IMC, CPMC, IPMC, C=contralateral; I=ipsilateral)) were calculated. Within each VOI maximum percent signal changes from rest to movement were found for simple and complex movement tasks (Figure 9.3). These values were put into a repeated measures GLM which revealed significant effects of task and hemisphere and significant interactions between brain region and task, and between hemisphere and task (Table 9.1). This test was followed up with t-tests to identify where differences occurred. The results from these tests are discussed below.

Factor	Levels		F	df	p
Within subject:					
Task	simple	complex	69.94	14	<0.001
Hemisphere	contralateral	ipsilateral	10.51	14	0.006
Brain region	MC	PMC			
Between subject:					
Hand	left	right	NS		
Interactions:					
Task and brain region			8.29	14	0.012
Task and hemisphere			11.04	14	0.005

Table 9.1: Repeated measures GLM of maximum % signal change in FMRI data. There were significant effects of task and hemisphere and significant interactions between task and brain region and between task and hemisphere. F values are Wilks' Lambda.

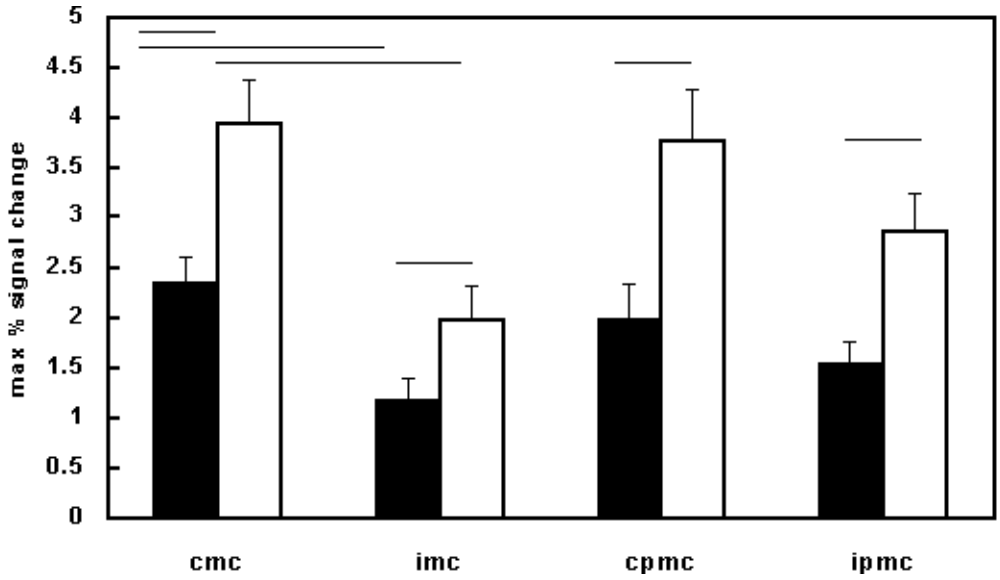

Figure 9.3: FMRI activation within volumes of interest during simple (black bars) and complex (white bars) movements. Horizontal lines indicate significant differences. There is a strong effect of complexity: for all VOIs there is significantly greater activation for the complex task. There are strong lateralisation effects in the motor cortex but not the premotor cortex: for both the simple and the complex task the contralateral MC is significantly more active than the ipsilateral MC.

The effect of task was present in all four VOIs (Figure 9.3), with significantly greater activation for the complex task in CMC (paired t-test, $t=6.2$, $df=15$, $p<0.001$), IMC ($t=5.2$, $df=15$, $p<0.001$), CPMC ($t=-7.0$, $df=15$, $p<0.001$) and IPMC ($t=-5.6$, $df=15$, $p<0.001$).

The difference in signal change between contralateral and ipsilateral cortical areas was greatest for the primary motor cortex (Figure 9.3). There was a significant difference in % signal change between CMC and IMC for both tasks (simple: t=3.10, df=15, p=0.007; complex: t=4.64, df=15, p<0.001), whereas there was no significant difference between CPMC and IPMC (simple task: NS; complex task t=1.83, df=15, p=0.088) (Figure 9.3).

The difference in signal change between the simple and complex tasks differed between brain areas and between hemispheres (Figure 9.4). This 'complexity effect' was significantly greater in CMC than IMC (t=4.461, df=15, p=<0.001), and significantly greater in IPMC than IMC (t=-3.276, df=15, p=0.005).

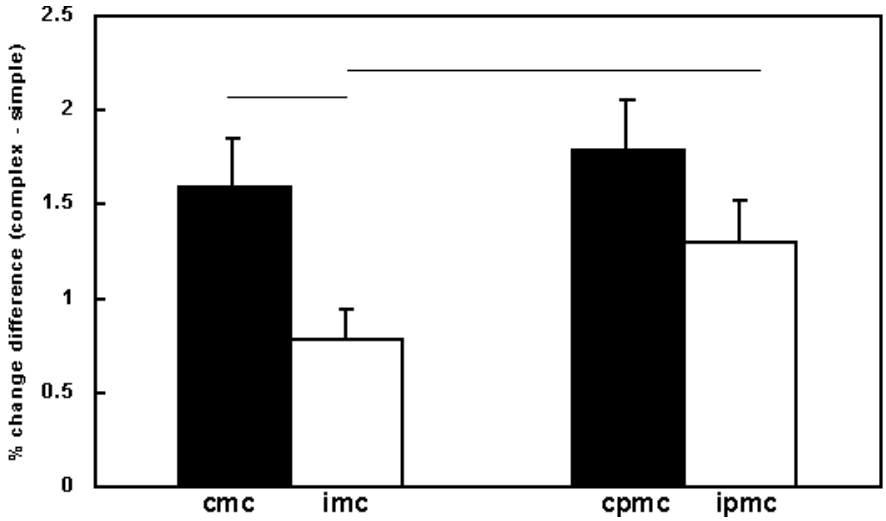

Figure 9.4: The size of the complexity effect (i.e.difference in FMRI activation during simple and complex movement tasks) within each VOI. Horizontal lines indicate significant differences. The effect is greater in ipsilateral premotor cortex (ipmc) than ipsilateral motor cortex (imc) and in contralateral motor cortex (cmc) than ipsilateral motor cortex (imc).

The GLM analysis found no differences in maximum % signal change between left or right hand movements (Table 9.1). Separating out the different conditions and VOIs there was no clear evidence for a difference in signal change between left and right hand movements (Figure 9.5).

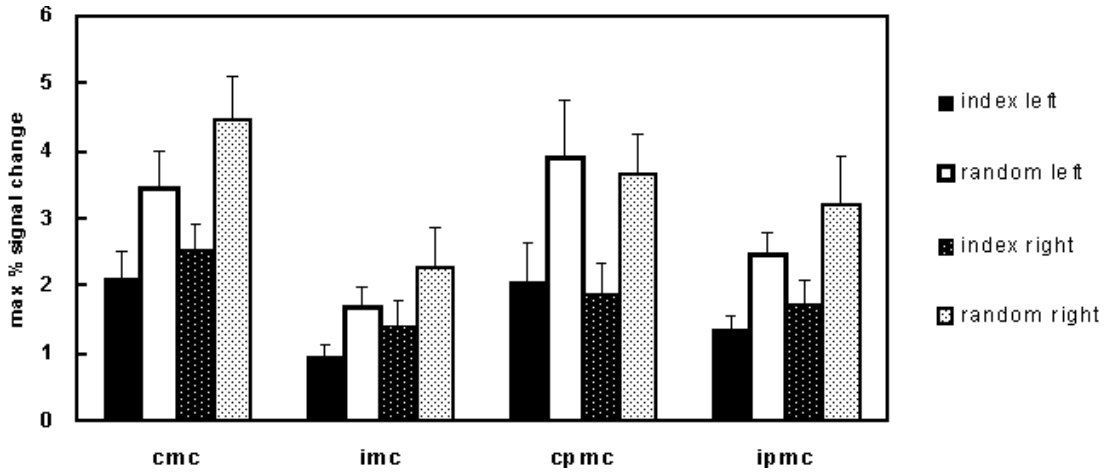

Figure 9.5: There was no evidence for significant differences in the maximum % FMRI signal change between the left and right hand groups for any VOI or for either task.

A laterality index was calculated for both sites (Figure 9.6). Laterality depended significantly on brain area but not on task: the motor cortex was significantly more lateralised than the premotor cortex for both the simple (paired t-test, t=5.00, df=15, p<0.001) and the complex task (t=3.86, df=15, p=0.002). There were no significant differences in laterality between the simple and the complex tasks.

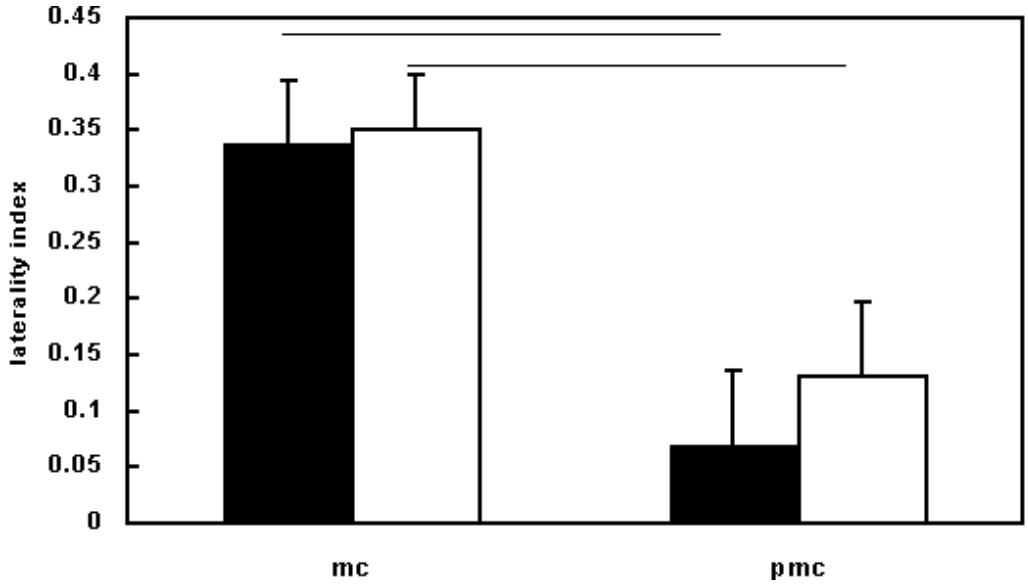

Figure 9.6: FMRI laterality index within VOIs for simple task (black bars) and complex task (white bars). There are no significant differences in laterality between the two tasks but MC activation is significantly more lateralised than PMC activation for both tasks. Horizontal lines indicate significant differences

TMS results

The functional significance of the ipsilateral activation detected with fMRI was tested in separate studies of the same subjects by applying TMS to temporarily interfere with processing in ipsilateral M1 and PMC during identical simple or complex finger movements.

Reaction times without TMS Reaction times without TMS were slower for the complex task (Table 9.2). Many subjects' reaction times for the simple task were less than 200ms, and therefore the TMS pulses at 200 and 250ms would not have affected their responses. These trials were therefore not analysed further.

Task	block	left hand group		right hand group	
		Mean	sd	mean	sd
Simple task	MC blocks	225.8	25.6	231.5	21.5
	PMC blocks	232.9	35.0	241.7	53.1
Complex task	MC blocks	409.6	45.5	399.7	58.5
	PMC blocks	406.1	39.6	407.2	74.7

Table 9.2: Raw reaction time values for left and right hand group for no-TMS trials during blocks of MC stimulation and PMC stimulation

Effect of TMS on reaction times Values for percent change in RT from a no-TMS baseline were calculated (Table 9.3). In order to test whether or not ipsilateral TMS of motor areas had a significant effect on reaction times a one-sample t-test was used to compare percent change values to zero. This was done separately for each task and each site at each time point, averaging across both subject groups (i.e. subjects moving left and right hands) (Figure 9.7). This showed that ipsilateral MC TMS produced a significant slowing of reaction times during simple tasks at 150ms ($t=3.349$, $df=15$, p corrected for 3 timepoints=0.012) and during complex tasks at 250ms ($t=3.405$, $df=15$, p corrected for 5 timepoints=0.02) (Figure 9.7). Ipsilateral PMC stimulation produced a significant slowing of reaction times during the simple task at 150ms ($t=2.923$, $df=15$, corrected

p=0.03) (Figure 9.7). The effects of ipsilateral PMC stimulation were not significant for the complex task. PMC stimulation speeded RTs when applied at 50ms during the simple task (t=-4.0, df=15, p=0.001). Thus when results were averaged across subjects performing the task with the left and the right hand, stimulation of ipsilateral MC or PMC slowed movements when applied late, in both simple and complex tasks.

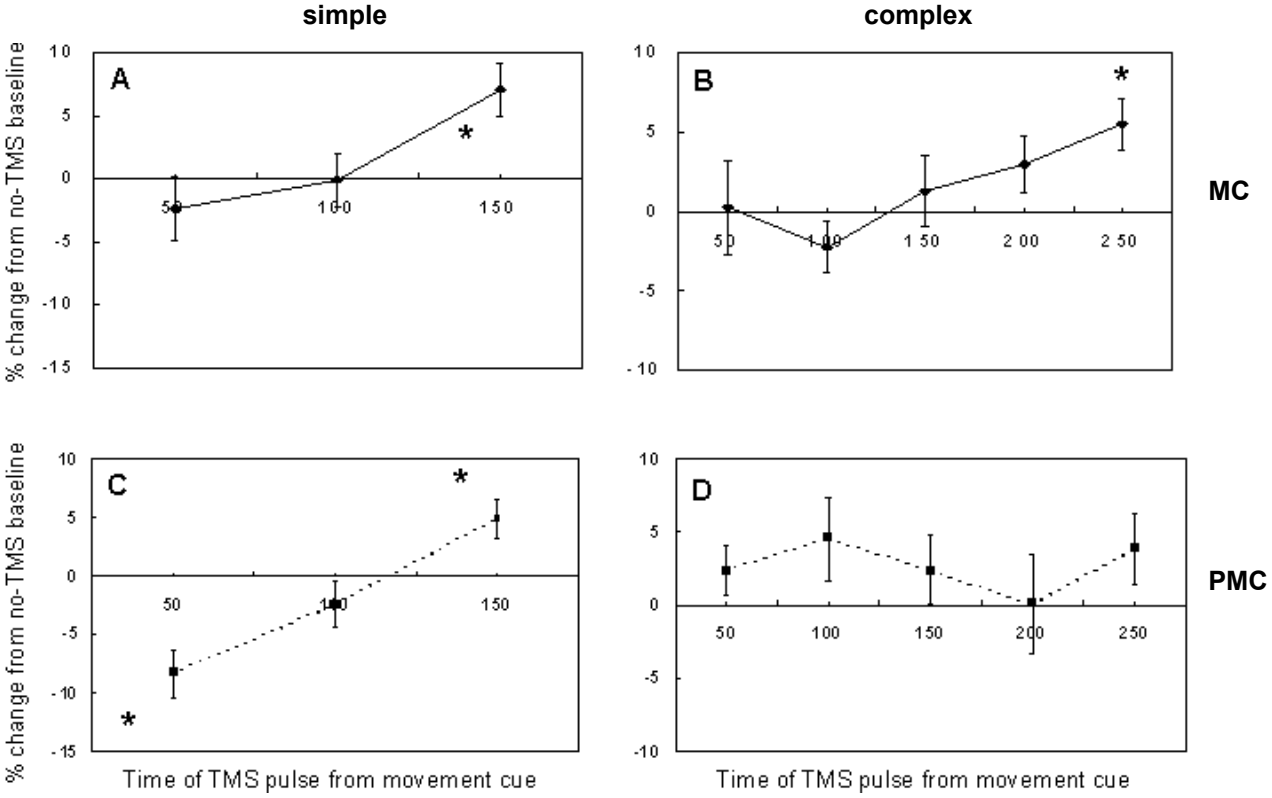

Figure 9.7: Effect of TMS on simple (left – A,C) and complex (right – B,D) tasks. TMS was applied over primary motor cortex (top - A,B) or premotor cortex (bottom - C,D) and different time points. Asterisks mark times when TMS produced a significant ($p<0.05$) slowing of reaction time from a no-TMS baseline. For simple movements data is given only for timepoints up to 150ms as many subjects' RTs were less than 200ms on this task and therefore results from TMS of later timepoints would not be meaningful.

To test for differences in the effects of TMS on different tasks, brain areas, stimulation times and hemispheres, values of % change in RT were analysed in a repeated measures GLM (Table 9.4). This revealed significant effects of time of TMS stimulation and hand being moved and interactions between task and brain region, between task and time, and between brain region, time and hand (Table 9.4).

Task	site	time	left hand group		right hand group	
			mean	se	mean	sd
simple task	MC	50	4.4	2.3	-9.1	2.6
		100	5.0	2.4	-5.2	2.0
		150	10.9	3.0	3.4	1.9
	PM	50	-7.2	3.0	-9.5	2.3
		100	1.1	2.3	-6.0	2.4
		150	5.6	2.0	4.2	2.4
Complex	MC	50	2.8	3.6	-2.3	4.0
task		100	-1.6	1.7	-2.9	2.5
		150	5.1	2.3	-2.6	2.9
		200	4.0	2.0	1.9	2.5
		250	8.4	2.4	2.6	1.2
	PM	50	1.1	2.2	3.6	2.0
		100	9.3	4.1	-0.3	2.2
		150	5.2	3.5	-0.5	2.2
		200	6.6	4.2	-6.5	3.4
		250	5.0	3.8	2.7	2.1

Table 9.3: Values of % change in RT from no-TMS baseline, for TMS of MC and of PMC at different time points and during the simple and the complex task. The group performing the tasks with the left hand received TMS over the left hemisphere; the group performing the task with the right hand received TMS over the right hemisphere.

Factor	Levels			F	df	p
Within subject:						
Task	simple	complex				
Time	50	100	150	20.486	13	<0.001
Brain region	MC	PMC				
Between subject:						
Hand	left	right		9.836	14	0.007
Interactions:						
Task and brain region				10.299	14	0.006
Task and time				5.083	13	0.023
Brain region, time and hand				5.701	13	0.017

Table 9.4: Repeated measures GLM for % change in RT from no-TMS baseline. There were significant effects of time and hand, and significant interactions between task and brain region, between task and time and between brain region, time and hand. The factor of time has only 3 levels: the data for 200ms and 250ms stimulation in the complex task could not be included as this data was not available for the simple task. F values are Wilks' lambda

Half the subjects performed the task with the right hand and half with the left hand. The significant effect of the hand being moved can be seen in Figure 9.8. TMS of both sites and during both tasks had a greater effect on ipsilateral hand movements when applied over the left hemisphere (Table 9.3, Table 9.5).

			t	P
simple task	MC	50	3.44	0.004
		100	2.89	0.01
		150	1.90	0.08
	PMC	50	0.55	NS
		100	1.95	0.07
		150	0.41	NS
complex task	MC	50	0.86	NS
		100	0.41	NS
		150	1.859	0.08
		200	0.56	NS
		250	1.96	0.08
	PMC	50	-0.74	NS
		100	1.84	0.09
		150	1.24	NS
		200	2.19	0.05
		250	0.49	NS

Table 9.5: Results from t-tests comparing the effects of TMS on left hand versus right hand movements. Note that different groups of subjects were studies for left and right handed movements. Subjects making left hand movements received stimulation over the left hemisphere and subject making right hand movements received stimulation over the right hemisphere. p values are uncorrected for multiple comparisons.

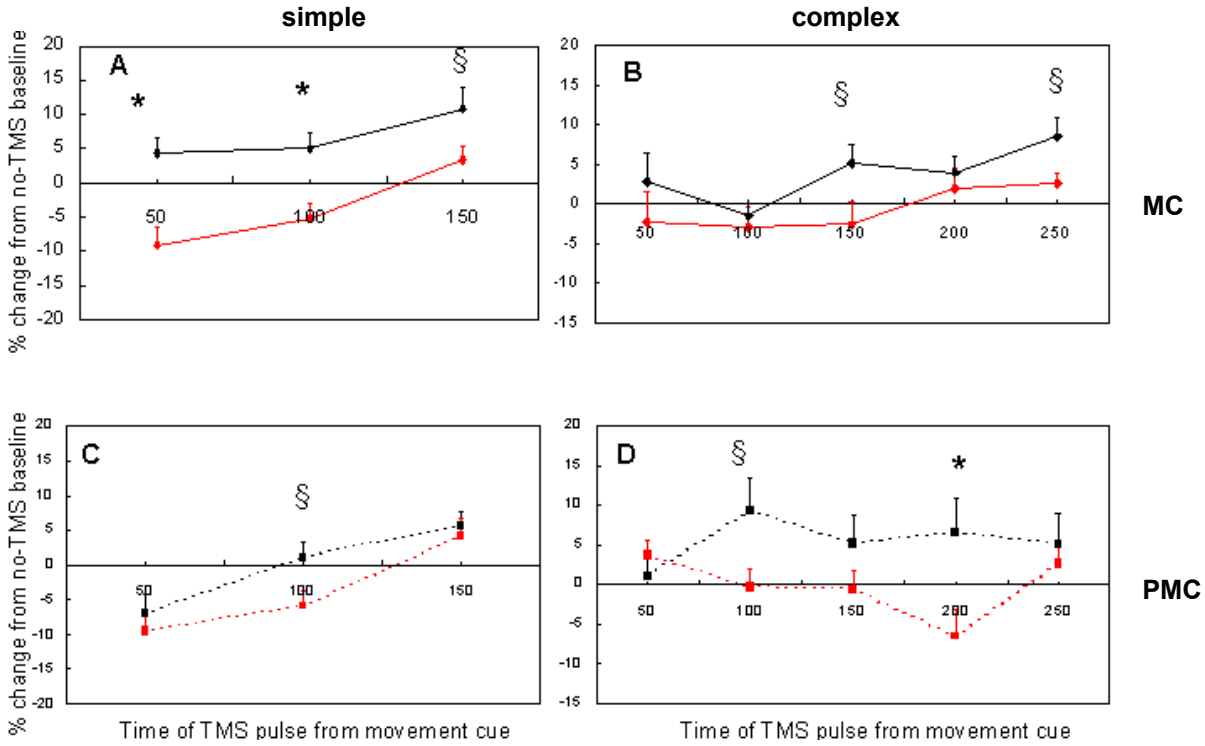

Figure 9.8: TMS had a greater effect on ipsilateral hand movements when applied over the left hemisphere (black lines) rather than the right hemisphere (red lines). Results for the simple task are shown on the left (A,C) and complex tasks on the right (B,D). Results for MC TMS are shown on the top row (A,B) and PMC TMS on the bottom row (C,D). Asterisks mark times when TMS produced a significantly ($p<0.05$) greater effect with left hand compared to right hand movements. § marks times where there was a trend ($p<0.15$) for a difference between left and right TMS.

The specific role of ipsilateral PMC (and not MC) early in complex movement is demonstrated in Figure 9.9. Averaged across all subjects, the effect of PMC TMS is significantly greater than the effect of MC TMS at 100ms in the complex task (paired t-test, t=-2.311, df=15, p=0.035).

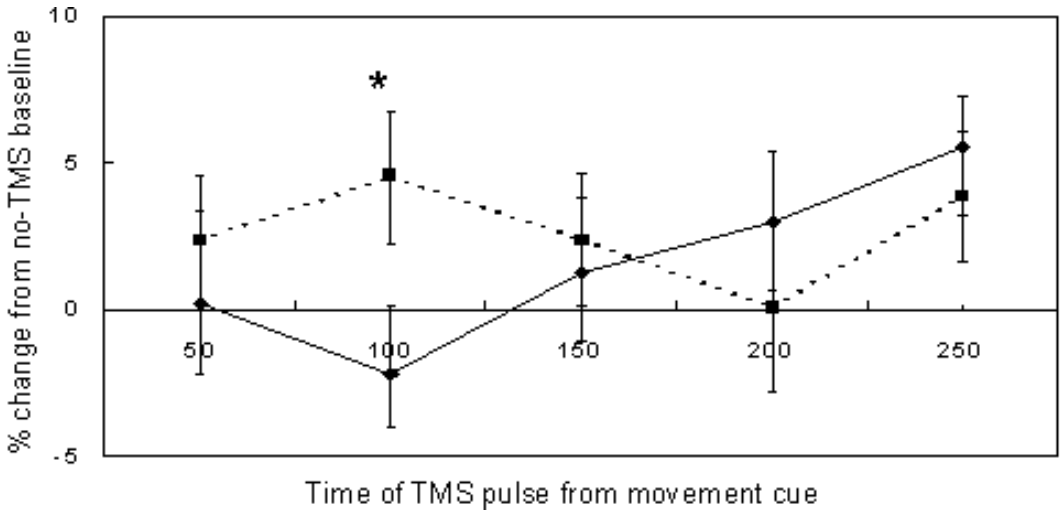

Figure 9.9: During complex movements there is an early time window during which ipsilateral TMS has an effect when applied over premotor (dotted line) but not primary motor (solid line) cortex. The asterisk marks the time (100ms) when TMS over the PMC produced a significantly ($p<0.05$) greater effect compared to stimulation over the MC.

The specific involvement of ipsilateral PMC early with complex (but not simple) movements is illustrated in Figure 9.10. Across all subjects, TMS of PMC has a significantly greater effect on complex compared to simple movements at 50ms (paired t-test, t=-3.880, df=15, p=0.001) and at 100ms (t=-2.583, df=15, p=0.021).

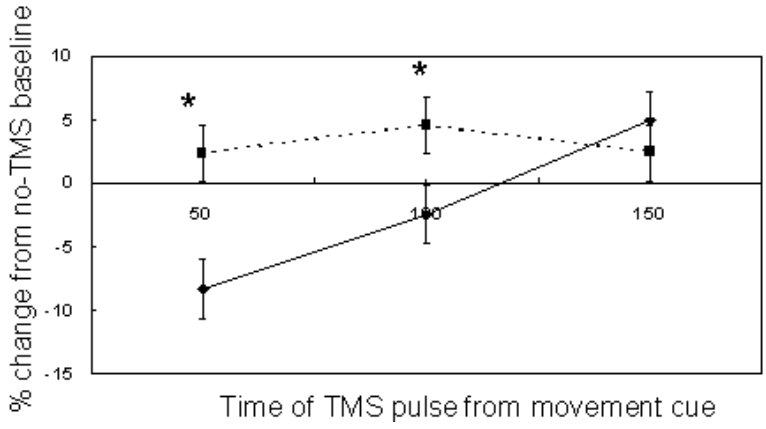

Figure 9.10: Early involvement of PMC is specific to complex movements (dotted line) rather than simple movements (solid line). Asterisks mark times when PMC TMS produced a significantly ($p<0.05$) greater effect during the complex task compared to the simple task.

MRI-guided localisation of TMS sites: MRI-guided localisation demonstrated that the trajectories of TMS targeted at the primary motor cortex (MC) site met the surface of the cortex around the central sulcus and overalapped well with the cluster of group FMRI activation in the primary sensorimotor cortex. The trajectories of TMS targeted at the dorsal premotor cortex site (PMC) met the surface of the cortex around the anterior precentral gyrus or superior precentral sulcus (Figure 9.11). These trajectories tended to be slightly anterior to the group FMRI activation in the dorsal premotor cortex. The initial scalp estimate of the premotor site was at 2 cm anterior and 1 cm medial to the motor hotspot. This site was defined based on previous literature (Schluter *et al.* 1998) and pilot studies, as producing dissociable effects from the MC site.

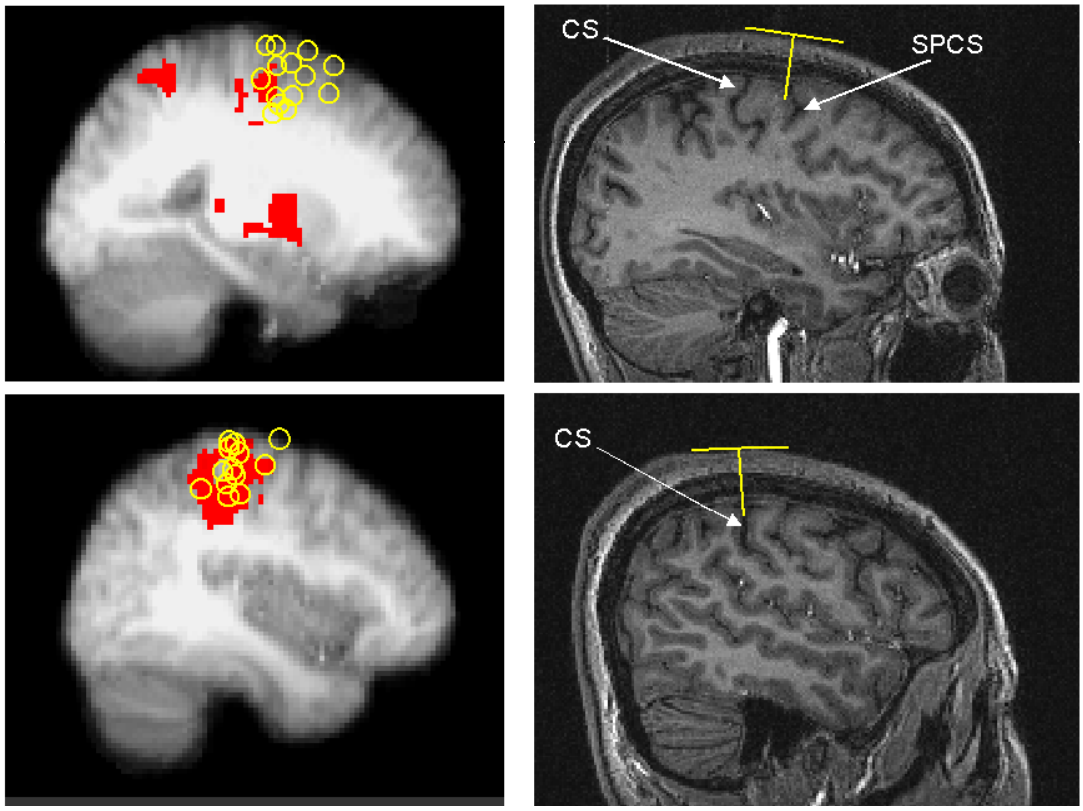

Figure 9.11: MRI guided localisation of sites of TMS stimulation.**Left:** TMS targets for all subject overlaid on the group FMRI maps. **Top:** targets for PMC site; **bottom:** targets for MC site. Saggital slices are chosen at the mean x co-ordinate for each site (PMC x=24, MC x=40). **Right:** Target sites in an individual subject for PMC (top) and MC (bottom). CS = central sulcus, SPCS = superior pre-central sulcus. FMRI maps are thresholded at Z>3.1 and significant clusters defined at p<0.01.

9.1.4 Discussion

Simple and complex movements produced changes in FMRI signal in motor cortical areas ipsilateral to the hand being moved. The role of these ipsilateral motor areas was tested by temporary interference with the same tasks using TMS.

9.1.4.1 Temporary interference with ipsilateral motor areas by TMS slows responses

Application of single TMS pulses at specific time points after a visual cue demonstrated not only which areas were important for different movements but also *when* processing in those areas was crucial. TMS of both sites was effective in significantly slowing movements at specific time points during the tasks (Figure 9.7). For both simple and complex tasks TMS of primary motor cortex (MC) or premotor cortex (PMC) slowed responses when applied late (150ms for simple task, 250ms for complex task). These results suggest that ipsilateral cortical motor areas play a crucial role even in simple hand movements.

An alternative interpretation of the late effects of TMS, common to both tasks and both sites, is that there is a non-specific disruptive effect of TMS for example, it may distract subjects if applied just before a response is about to be made, or it may simply inhibit the opposite hemisphere. However, this interpretation is not supported by the finding of significant differences between TMS of the left and right hemisphere (Figure 9.8). If the slowing effects of late TMS were due to general distraction or inhibition, then equal effects would be expected for stimulation of the left and right hemispheres. However, there are clear differences between TMS of different hemispheres. For example, late TMS of ipsilateral MC has a much greater effect when applied over the left hemisphere than the right (Figure 9.8a, b).

In addition to the significant effect of side of stimulation there were also effects that were site-specific and task-specific. TMS of either site at late timepoints slowed

responses in both tasks. However, early TMS of left PMC also slowed responses in the complex task. This early effect was not seen with right PMC stimulation (Figure 9.8d) or with MC stimulation (Figure 9.9) and was not seen with left PMC stimulation during the simple task (Figure 9.10). This result suggests that left premotor cortex has a specific role in the early stages of complex cued movements.

The different effects of MC and PMC stimulation also demonstrate that the effects of TMS were spatially specific. This is important to establish as it is well known that the current induced by a single TMS pulse spreads to adjacent and interconnected areas of cortex (see Section 3.2.2.1). However, it is apparent that with appropriate levels of stimulation, and use of a figure-of-eight coil, stimulation of scalp sites separated by 1-2 centimetres can produce dissociable behavioural effects.

Some of the findings from this section are consistent with those from an earlier single pulse TMS study (Schluter *et al.* 1998). Both this section and the study by Schluter et al (1998) reported a left hemisphere dominance for the control of ipsilateral hand movements. Both studies also found an effect of early TMS (100ms in the current study, 140ms in Schluter et al, 1998) that was specific to complex movements (choice reaction time versus simple reaction time in the Schluter et al study) and to the left premotor cortex. However, there are some differences between the findings of the two studies. In particular, there was no effect of TMS of the ipsilateral motor cortex in the study by Schluter et al, whereas this section found that MC TMS caused significant slowing of responses in both simple (at 150ms) and complex (at 250ms) tasks. However, there are also clear differences in design between the studies and so the results may not be directly comparable. For example, the current study used visually-cued index finger pressing (simple task) versus visually-cued random finger pressing (complex task). In both tasks the visual cue was a schematic representation of the hand, and there was a clear mapping between positions on the schematic hand and responses. In the Schluter et al study the

tasks were simple reaction time (press index button whichever cue appears) and choice reaction time (for large rectangles or small circles press index button, for small rectangles or large circles press middle button). Therefore in the Schluter et al study the association between cue and movement had to be learnt and there was no simple spatial mapping between the cue and the movement. The processing steps between visual cue and movement execution would therefore have differed between the two designs. Learnt, non-standard mappings between cue and movement are known to particularly involve the premotor cortex (Grafton *et al.* 1998; Wise *et al.* 1996) and therefore the relative importance of the ipsilateral primary motor cortex function in determining overall reaction times may have been reduced in these tasks.

It is difficult to directly compare the findings of the current study with those of the study by Gerloff et al (1998) as they used repetitive TMS whereas the current study used single pulse TMS. However, there was a suggestion from their study that stimulation of ipsilateral motor and premotor areas slowed finger movements, and that the effect of ipsilateral PMC stimulation was enhanced for complex sequences (see figure 9.1 and introduction). In the current study, TMS of the ipsilateral PMC had a significantly greater effect on complex movements than simple movements (Figure 9.10). There was also a significant interaction between time and task, suggesting that the size of the complexity effect depended on the time of the TMS pulse. This finding provides evidence for the suggestion that the Gerloff et al study (which used repetitive TMS) cannot be directly compared to the results presented here.

9.1.4.2 Relationship between TMS and FMRI results

TMS was targeted close to areas of increased FMRI signal change. The FMRI signal change in motor areas during complex movements was significantly greater than that found during simple movements in all regions tested (see Figures 9.2 and 9.3). This is

consistent with results from the pilot study reported in Section 5. In the primary motor cortex, the increase in signal with complexity was greater in the contralateral than ipsilateral hemisphere (Figure 9.4). This is reflected by a slight increase in primary motor cortex laterality index with complex movements (Figure 9.6). In the ipsilateral hemisphere the increase in signal with complexity was greater for the premotor than the primary motor cortex (Figure 9.4).

There was considerable agreement between the results from the FMRI and TMS studies of the same movement tasks. Both the TMS and the FMRI results indicate significant involvement of ipsilateral motor areas in simple and complex hand movement. Both approaches showed that increasing complexity led to greater increases in premotor cortex involvement than primary motor cortex involvement. With the temporal resolution of TMS it is possible to determine the time points at which this increase is greatest (i.e. 50-100ms, Figure 9.10).

However, there are also some apparent discrepancies between the results from the TMS and the FMRI experiments. For example, whereas the TMS results showed a very clear left hemisphere dominance for involvement in ipsilateral hand movements with the group performing left hand movements showing greater disruption effects (Figure 9.8), there was no evidence from the FMRI data to suggest that the same subjects had a greater signal change in ipsilateral motor cortical areas with left hand movement (Figure 9.5).

One possible explanation for the lack of a difference in the FMRI experiment could be lower statistical power in the FMRI study. However, the same numbers of subjects participated in the two studies. Assuming that the true difference between left and right handers would be of the same magnitude with the two techniques, the remaining determinant of power would be the standard deviation of the measurements relative to the size of the measurements (Colton 1974). In the TMS study, the standard

deviation in percent change in reaction time for the different conditions ranged from 1.1 to 1.8 times the mean percent change (Table 9.3). In the FMRI study the standard deviation of percent signal change ranged from 0.5 to 0.6 times the mean percent signal change (Figure 9.3). Given these facts, the FMRI study should not have had lower statistical power than the TMS study.

Therefore the discrepancies between the TMS and FMRI results may reflect differences in the type of information available from the two techniques. First, there is a difference in temporal resolution between the two approaches. As demonstrated by the results presented there, dissociable effects of TMS can be achieved with pulses 100ms apart. By contrast the temporal resolution of BOLD FMRI is limited by the variability and sluggishness of the haemodynamic response. Therefore while the TMS data applies to specific discrete time points in the movement, the FMRI results reflect activation averaged across all stages of each cued finger movement and intervals between finger movements. The results from the TMS experiment demonstrates that certain effects are only apparent within limited time windows.

In addition the two techniques differ in the physiology that is being investigated. The BOLD signal mainly reflects pre-synaptic processes, and therefore a local increase in BOLD may indicate increased activity in areas that project to the region of interest rather than increased neuronal firing in the area where increased BOLD is detected (Logothetis *et al.* 2001). By contrast, local application of TMS predominantly effects underlying neurons and therefore influences their output directly. However, both simultaneous measurements of electrical and haemodynamic changes and biophysical modelling suggest that BOLD changes do also correlate with spiking output, albeit to a lesser degree (Logothetis *et al.* 2001; Rees *et al.* 2000).

The spatial distribution of areas imaged by FMRI and those stimulated by TMS also differs due to current spread. Estimates of the decay of the induced current over

distance suggest that there will be minimal stimulation of neurons deep in the central sulcus (Roth *et al.* 1991) where hand-related neurons whose haemodynamic supply is detected by FMRI might be located (Penfield and Boldrey 1937). However, the TMS-induced current will spread transynaptically to adjacent and interconnected regions (Ilmoniemi *et al.* 1997).

The mismatch between the outcomes of TMS and FMRI regarding left dominance may echo the differences often observed between activations detected in brain imaging studies and the effects of permanent lesions. For example, while language deficits typically result only from left hemisphere damage, imaging studies of activation by speech sounds tend to identify bilateral areas of activation (Binder *et al.* 2000). This discrepancy seems to depend on the choice of control task. Only when the acoustic properties of speech are controlled for and intelligibility is isolated is activation found specifically lateralized in the left anterior superior temporal sulcus (Scott *et al.* 2000). Therefore in the experiment presented in this subsection, the comparison of movement tasks to a rest baseline may not have well isolated the left hemisphere differences.

In contrast to the results reported in Section 5, increasing complexity in the experiment presented here was not associated with the more bilateral pattern of motor activation. The reasons for this discrepancy are not clear. The effect in Section 5 was not driven by the left handed subjects in that study as they showed only very small decreases (2 subjects) or even increases (1 subject) in laterality with increasing complexity. The complex tasks in the two studies were identical but the simple tasks differed between the two studies (index finger tapping in this section and sequential four finger tapping in section 5). Another difference between the two studies is that whereas subjects in the experiment presented performed one practice block of the task before entering the magnet, subjects in the experiment in section 5 did not practice the task and therefore may have found the task more effortful.

9.1.4.3 Conclusions

This subsection demonstrated that simple and complex movements led to changes in FMRI signal intensity in the primary and premotor cortex ipsilateral to the hand moved. Temporary interference with these areas using TMS produced a slowing of reaction times, suggesting that the processing in those regions was crucial for normal task performance. There were some discrepancies between the slowing effects of TMS and the signal change detected by FMRI. Further approaches to assessing whether the slowing effect of ipsilateral TMS correlates with the laterality of FMRI signal changes are explored in the next section.

9.2 The effect of ipsilateral motor cortical TMS on movement of the affected hand in patients with stroke

The previous section demonstrated that simple and complex movement tasks activate the ipsilateral motor and premotor areas in normal control subjects. In addition, temporary interference with these areas using TMS slows reaction times. However, the precise relationship between FMRI signal changes and the slowing effects of TMS was unclear. This may be due to the different sensitivities of the two approaches. In addition, the range of relative TMS and FMRI effects was limited in this group of normal controls, making it difficult to define any correlations between the two variables. We therefore chose to study a group of stroke patients, with varying degrees of motor impairment, in order to test the significance of ipsilateral FMRI activation. It was hoped that patients would show a wide range of laterality effects in the FMRI signal, and a wide range of TMS-induced slowing effects, thereby allowing a more powerful assessment of the relationship between the two. In addition, comparing the magnitude of TMS-induced slowing effects in patients relative to controls could provide a test of the hypothesis that there is increased recruitment of ipsilateral motor areas after stroke.

9.2.1 Introduction and rationale

Although TMS has been used extensively in the investigation of motor representations after stroke (Turton *et al.* 1996; Cicinelli *et al.* 1997; Rossini *et al.* 1998; Traversa *et al.* 1997; Traversa *et al.* 2000; Netz *et al.* 1997), it has not previously been used as a temporary interference technique to investigate the importance of different motor areas in recovered movements.

Previous studies have used TMS to map the extent and location of areas of cortex where stimulation elicits muscle responses (Turton *et al.* 1996; Netz *et al.* 1997). This approach has provided evidence for increased efficacy of uncrossed motor pathways

after stroke as ipsilateral muscle responses to motor cortex TMS are more common in stroke patients than in controls. Such responses are most common in poorly recovered patients (Turton *et al.* 1996; Netz *et al.* 1997) suggesting that recruitment of uncrossed pathways is maladaptive, or alternatively that ipsilateral pathways are recruited in situations where few alternative options are available (i.e damage to the crossed spinal tracts is extensive).

The present study used TMS to temporarily interfere with ipsilateral motor cortical processing during movements of the affected hand in stroke patients. The aim of this study was two-fold. Firstly, by recruiting patients with a range of motor impairments, high variability in TMS and FMRI measures might be expected. Therefore, this subject group could provide further insight into the relationship between the TMS and FMRI measures. Secondly, comparing stroke patients to control subjects could reveal whether the effects of ipsilateral TMS are enhanced in stroke patients, given the imaging evidence for increased activity in the ipsilateral motor cortices.

9.2.2 Methods

Subjects: 11 right-handed patients (age 50.4 $\pm$ 11.14) after first ischemic left middle cerebral artery (MCA) stroke (Table 9.6) were compared to a subset of 5 of the control subjects from Section 9.1 who were adequately age-matched (age 48.4 $\pm$ 14.2). Stroke volume varied between patients (Table 1), but all patients were in a clinically stable recovery period following first presentations with unilateral hemiparesis. No cortical strokes involved the hand area of M1 (Yousry *et al.* 1997) or dorsal PMC (Rizzolatti *et al.* 1998). A hand impairment measure was calculated for each patient based on reaction times for visually cued fixed index finger tapping (impairment = ((A-U)/U)*100, where A=affected, U=unaffected hand)

Patient	Sex	Age	Volume	Time post stroke	Impairment score
1	M	59	<0.1	40	10.9
2	F	57	8	12	3.8
3	M	45	36	24	-6.4
4	M	55	4	12	-0.4
5	M	50	12	13	17.6
6	F	35	0.15	5	12.7
7	M	42	<0.1	8	-4.1
8	M	53	<0.1	31	1.5
9	M	75	<0.1	7	5.7
10	M	44	<0.1	4	17.9
11	M	46	<0.1	12	-6.1

Table 9.6: Clinical details of left middle cerebral artery strokes in patients studied. Lesion volume is in cm^3. Time post stroke is in months. Impairment score is the difference between affected and unaffected hand reaction time for visually-cued fixed finger button press (without TMS). M=male, F=female.

FMRI scanning, image analysis and TMS as for Section 9.1. All patients could perform the simple task (fixed index finger tapping) well. Some patients were unable to perform the complex task (randomly cued finger tapping) task and those who did perform that task did so more slowly than controls (patients, 550 $\pm$ 55 ms; age-matched controls, 423$\pm$36 ms). For this reason data from the simple task only are reported for patients.

Volumes affected by excessive motion (>10mm displacements) were discarded. This procedure was necessary for two patients (patients 7 and 9). For one patient (patient 8) excessive motion was present throughout the experiment and therefore all data from this patient were discarded.

Statistics: Within group comparisons were performed as for Section 9.1. In addition, comparisons were made between stroke patients and healthy controls using repeated

measures GLMs. Data from the most effective timepoints for TMS, as defined by the control experiment, were analysed further. 95% confidence intervals were found for the control group for percentage change in reaction time in the TMS experiment, and for maximum percent signal change in the FMRI experiment. Patients falling outside these limits were identified. Correlations between FMRI and TMS results were tested.

9.2.3 Results

FMRI results Like controls, patients activated a network of sensori-motor areas during hand movement. Analysis of signal changes within VOIs during fixed index finger movements showed that there were large variations in the magnitude, extent and laterality of FMRI activations among patients (who were heterogeneous for lesion volume and for residual functional impairment) (Figure 9.12). In consequence, there were no significant group differences between patients and controls.

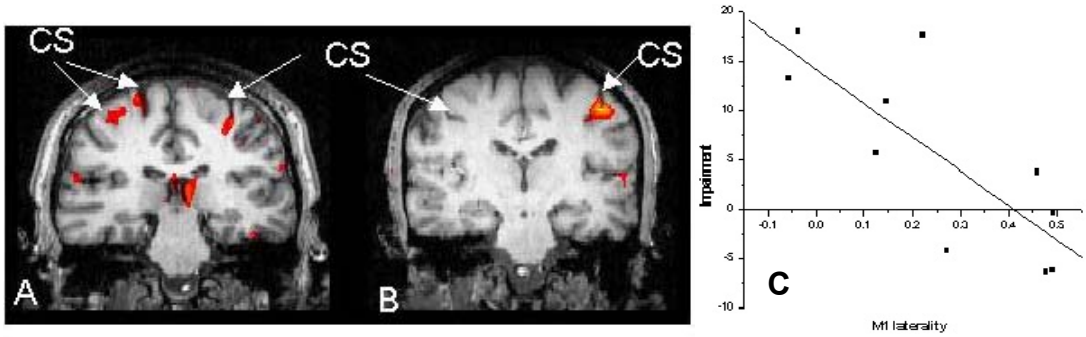

Figure 9.12: There was variability in the laterality of FMRI activation in patients. Figure shows representative activation maps for fixed finger tapping versus rest for two individual patients. Bilateral motor cortex activation was most common in more impaired patients (e.g A, impairment score=17.9). Predominantly contralateral activation (i.e. similar to the control pattern) was most common in less impaired patients (e.g. B, impairment score = -6.2). We found a correlation between impairment and laterality (C). FMRI data are thresholded at $Z>3.1$, and a cluster extent threshold of $p<0.01$.

The relationship between FMRI measures and hand impairment was tested. Relatively increased ipsilateral activation, reflected by lower laterality indices, was most common in poorly recovered patients; there was a negative correlation between impairment and fMRI laterality in M1 ($r=-0.79$, $p=0.007$, Figure 9.12) and a trend for a correlation with laterality in PMC ($r=-0.55$, $p=0.1$).

TMS results The functional significance of ipsilateral activation in patients was tested by TMS-induced disruption of simple index finger movements in between patients and controls.

Without TMS, reaction times for simple finger tapping in patients (240.7 $\pm$ 48.3 ms) were similar to age-matched controls (228.9 $\pm$ 23.5 ms).

The relative change in RT with ipsilateral TMS applied to both M1 and PMC varied with the time of stimulation relative to the cue (M1: F=9.91, p=0.001; PMC: F = 5.57, p=0.01). For TMS of iPMC there was also a significant interaction between stimulation time and group (F=4.84, p=0.016), suggesting that the differences between patients and controls were stimulation time dependent. The effects of iPMC TMS were greater for patients than for controls when applied 100ms after the cue to move (Figure 9.13).

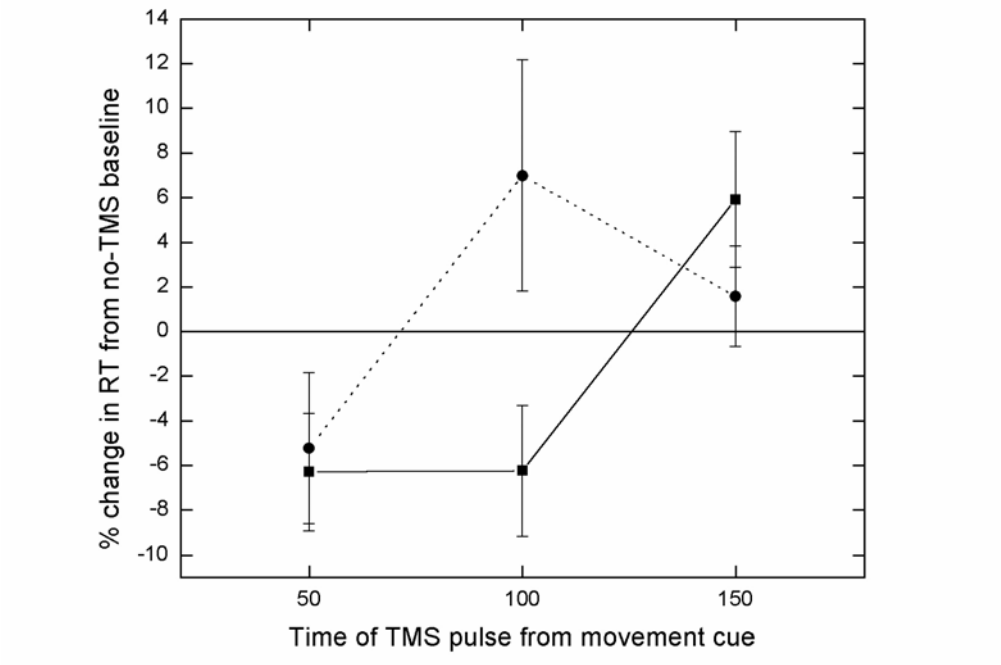

Figure 9.13: TMS over iPMC during simple movements had distinct effects in patients (dotted line) and controls (solid line). Pulses at 100ms slowed patients but not controls. This early slowing effect of iPMC TMS was only seen in controls during randomly cued movements (see Figures 9.9 and 9.10).

This pattern of iPMC involvement in patients is similar to the pattern seen in control subjects during complex movements (see Figures 9.9 and 9.10). The results suggest that at least some of the patients are recruiting iPMC early after the cue to move.

Correlations between TMS interference and FMRI or hand impairment. The extent to which recruitment of iPMC at this early time reflected patterns of activation present in the fMRI data for patients was tested. There was a negative correlation between the magnitude of the 100 ms iPMC TMS effect and FMRI laterality index in PMC (r=-0.82, p=0.004, Figure 9.14). This suggests that the laterality of FMRI signal changes reflect functionally significant activity.

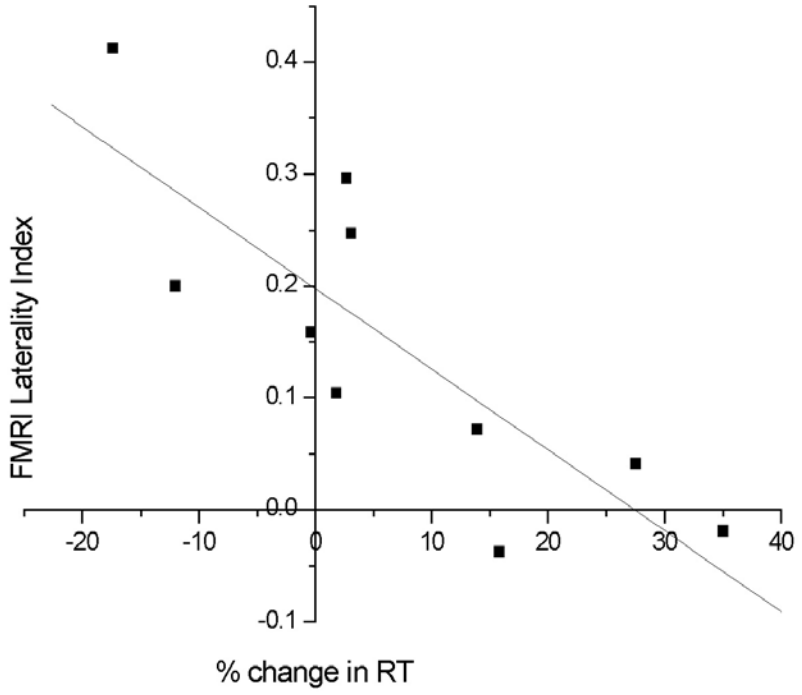

Figure 9.14: In patients with left hemisphere stroke there was a significant correlation between TMS and FMRI measures. Patients with a low PMC FMRI laterality index (i.e. relatively bilateral) during simple movements also showed a large slowing effect of 100ms iPMC TMS during simple movements.

Finally, the relationship between the magnitude of TMS effects was related to the degree of hand impairment was tested. There was a positive correlation between the effect of iPMC TMS at 100ms and a measure of finger movement impairment in patients that was close to significance (r=0.62, p=0.057).

MRI-guided localisation of TMS sites: MRI-guided localisation demonstrated that there was overlap in the TMS trajectories for patients and controls (Figure 9.15). For

both groups of subjects, the trajectory of TMS of the MC site was targeted at the central sulcus; the trajectory of TMS of the PM site was targeted at the precentral gyrus/superior precentral sulcus (Figure 9.15).

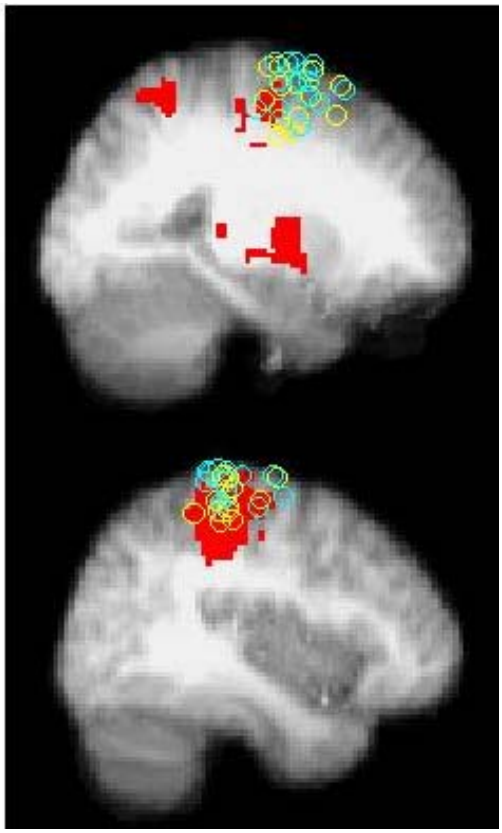

Figure 9.15: TMS target for patients (blue) and controls (yellow) overlapped for PMC (top) and MC (bottom) sites

9.2.4 Discussion

FMRI scanning of patients after stroke demonstrated that patterns of brain activity alter after injury; brain activation during fixed finger movements is more bilateral in more severely impaired patients. However, it is uncertain whether the ipsilateral, motor related activity is behaviourally significant. TMS was therefore used to test the functional significance specifically of this activation.

Interference with ipsilateral motor areas by TMS slowed movements in both healthy controls and patients, but interference in patients occurred in a manner distinct from that seen in the healthy controls. The differential effect of TMS in patients and controls depended on pulse location and timing. TMS of right PMC slowed right index

finger movements in right hemiparetic patients and not controls when applied 100ms after the cue to move. This suggests that the increased ipsilateral motor cortex activation observed in patients after strokes causing hemiparesis reflects functionally significant recruitment of this cortex in the motor task. The magnitude of the 100ms iPMC TMS slowing effect in patients correlated with the laterality of PMC fMRI activation during fixed finger movements, so fMRI activity lateralisation change after stroke reflects this adaptive recruitment of ipsilateral cortex. The magnitude of the 100ms iPMC TMS slowing effect in patients correlated with impairment, suggesting that poorly recovered patients depend more on iPMC to perform fixed finger movements.

The finding that the effects of TMS in the stroke group depend on the timing of the TMS pulse demonstrates, however, that increased recruitment of ipsilateral motor areas is important only during specific periods of movement processing. The possibility that iPMC plays a specific role early in simple movements after stroke is interesting given that similar interference timing was observed for randomly cued movements in control subjects here and in previous studies of a choice reaction time task (Schluter *et al.* 1998). This role appears to be unique to the injured brain, as, in healthy controls, while early (50-100ms) TMS of iPMS slowed randomly cued movements, it did not affect index finger movements. Execution of even simple movements with an impaired limb may involve a spatial and temporal pattern of motor cortical recruitment that is normally associated with more complex movements.

The TMS results presented in this subsection suggest that it is primarily the premotor rather than the primary motor cortex that is recruited for simple movements in more impaired patients after stroke. Consistent with this, previous imaging studies have reported increased activation in iPMC during movement of the affected limb (Weiller *et al.* 1992) and the present study has shown that such activations tend to be found in more impaired patients (Seitz *et al.* 1998). This could reflect increased recruitment of

uncrossed corticospinal projections from the dorsal premotor cortex. Although the majority of corticospinal projections originate in M1 a substantial proportion come from other motor areas (Dum and Strick 1991). In addition, while 70-90% of pyramidal fibres decussate into the lateral corticospinal tract, 10-30% are uncrossed and descend as the ventral corticospinal tract (Nathan and Smith 1973). The dorsal PMC has prominent bilateral connections to the spinal cord (Kuypers and Brinkman 1970). However, the ipsilateral connections of PMC are with ventromedial spinal areas that are less concerned with distal movement.

The pattern of spinal connectivity from PMC therefore may constrain the degree of recovery possible. The more impaired patients, in whom iPMC appeared to be most important for movement, were able to make the simple movements needed for the fixed task, but were unable to make the individual finger movements required for the complex task. Spinal connections made by non-primary motor areas are different to those made by M1 (Maier *et al.* 2002). This may also limit the extent and manner in which PMC can contribute to recovery. While the present results demonstrate that the iPMC may contribute to movement recovery in certain patients, they cannot be interpreted as showing that one area is functionally substituting for another in a complete and simple way (Fries *et al.* 1993). Rather, iPMC behaves as if it mediates partial adaptive compensation for injured motor cortex after stroke. Further experiments are needed to clearly establish the exact anatomical route by which the ipsilateral premotor cortex influences the spinal cord in these patients.

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