

**Observing Actions After Stroke: Investigating  
the Potential of the Mirror Neuron System as a  
Rehabilitation Tool**

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## Overview

Motor impairments are a common consequence of stroke, but some patients are too impaired to participate in physiotherapy. In primates, a set of neurons have been identified that are active during both performance and observation of motor tasks. A similar “mirror neuron system” (MNS) in the humans could potentially be utilised to augment physiotherapy in stroke patients, by merely observing another’s actions.

In Part 1, evidence for the existence of a human MNS is reviewed. There is a wealth of converging evidence to support the existence of a mirror system in healthy individuals, consisting of a distributed network of brain regions that are activated by the observation of others’ actions. Furthermore, activation of the MNS can facilitate motor output, and preliminary findings suggest that action observation may improve motor performance in stroke patients.

Part 2 reports a behavioural and brain imaging study that aimed to investigate the MNS in stroke patients, in order to establish its potential as a rehabilitation tool. Two experiments were carried out using a 3x3 mixed factorial design. Stroke patients, age-matched healthy volunteers and younger healthy volunteers participated in a “one-back task”, in which they observed and responded to video clips of hand movements (pinch, grasp and non-goal directed movements). Eight stroke patients, 11 age-matched controls, and 16 younger controls participated in Experiment 1, in which accuracy rates on the task were recorded. In Experiment 2, eight stroke patients, nine age-matched controls and 11 younger controls performed the one-back task during functional magnetic resonance imaging (fMRI). In the healthy participants, there was a positive correlation between task accuracy and activity in

left premotor and inferior parietal cortices, implying the one-back task may be a novel behavioural correlate of MNS activity. Behavioural and fMRI results indicated that MNS activity was present in the stroke patients. However, there was a reduction in MNS activity compared to controls, particularly for the observation of more functionally impaired movements (pinch). No age-effects were found in either experiment. These findings suggest that action observation has potential as an intervention for stroke patients across the adult age range. Its utility as a rehabilitation tool may be dependent on patients' existing motor abilities, and the clinical implications of this are discussed.

The final part of the thesis is a critical reflection on the research process. The development of the research question, methodological dilemmas, reflections on the research process, strengths and limitations of the research, future directions and further clinical implications are discussed.



## Table of Contents

### Page

<b>Part 1: Literature Review</b> .....	11
<b>Can the observation of another's actions enhance the motor performance of stroke patients? A review of the mirror neuron system literature and its implications for stroke rehabilitation.</b>	
Abstract.....	12
<b>1. Introduction</b> .....	13
<b>2. Background</b> .....	14
2.1 Neuroplasticity following stroke.....	14
2.2 Treatment of stroke patients with upper limb motor impairments.....	17
2.3 The mirror neuron system – an alternative approach to rehabilitation?...19	
<b>3. Review of converging evidence for the existence of a human mirror system, and its implications for stroke rehabilitation</b> .....	22
3.1 Methods.....	22
3.2 Transcranial magnetic stimulation (TMS) studies.....	22
3.3 Respiration rates.....	26
3.4 Behavioural studies .....	28
3.5 Clinical neuropsychological evidence.....	30
3.6 Electroencephalography (EEG) studies.....	31
3.7 Brain imaging studies.....	33

**Page**

3.8 Stroke rehabilitation.....	43
<b>4. Conclusions.....</b>	<b>47</b>
<b>5. References.....</b>	<b>48</b>
 <b>Part 2: Empirical Paper.....</b>	 <b>59</b>
 <b>Observing Actions After Stroke: Investigating the Potential of the Mirror</b>	
<b>Neuron System as a Rehabilitation Tool</b>	
 Abstract.....	 60
<b>1. Introduction.....</b>	<b>61</b>
1.1 The consequences of stroke.....	61
1.2 The Mirror Neuron System (MNS).....	61
1.3 The MNS as a rehabilitation tool.....	64
1.4 Aims.....	65
1.5 Hypotheses.....	66
<b>2. Methods.....</b>	<b>69</b>
2.1 General Methods.....	69
2.1.1 Participants.....	69
2.1.2 Stimuli.....	71
2.1.3 One-back paradigm.....	73
2.1.4 Training.....	75
2.1.5 Motor functioning measures.....	76
2.1.6 Measure of spatial attention and working memory.....	77
2.1.7 Design .....	78

**Page**

2.2 Experiment 1 – Behavioural Study.....	79
2.2.1 Power calculation.....	79
2.2.2 Participants.....	80
2.2.3 Procedures.....	80
2.3 Experiment 2 – fMRI Study.....	81
2.3.1 Power calculation.....	81
2.3.2 Participants.....	82
2.3.3 Procedures.....	82
2.3.4 Data acquisition.....	84
2.3.5 Data pre-processing.....	85
2.3.6 Statistical analysis.....	86
<b>3. Results.....</b>	<b>87</b>
3.1 Experiment 1 - Behavioural Study.....	87
3.1.1 Accuracy rates.....	87
3.1.2 Response times.....	89
3.1.3 Spatial Span.....	90
3.1.4 Motor function tests.....	91
3.2 Experiment 2 – fMRI Study.....	94
3.2.1 Healthy volunteers.....	94
3.2.2 Patients.....	103
3.2.3 Between-groups comparisons.....	108
<b>4. Discussion.....</b>	<b>114</b>
4.1 Behavioural findings.....	114
4.2 fMRI findings.....	119

**Page**

4.2.1 Healthy volunteers.....	119
4.2.2 Patients.....	124
4.3 Summary and conclusions.....	128
<b>5. References.....</b>	<b>130</b>

**Tables and Figures**

<b>Table 1</b> Patient characteristics.....	70
<b>Figure 1</b> Example still images of the video stimuli.....	73
<b>Figure 2</b> Behavioural testing and fMRI paradigm.....	75
<b>Table 2</b> Mean percentage accuracy rates.....	89
<b>Table 3</b> Motor functioning scores.....	91
<b>Figure 3</b> SPM {Z}s representing brain activity for observing either goal directed (top) or non-goal directed actions in the healthy controls. ....	96
<b>Table 4</b> Observing goal directed versus non goal directed actions.....	97
<b>Figure 4</b> SPM {Z}s representing relative increases in brain activity during the observation of goal directed actions compared to non-goal directed actions in the control group.....	98
<b>Figure 5</b> Prefrontal cortex more active during observation of goal directed actions in controls.....	99
<b>Figure 6</b> Parameter estimates (magnitude of brain activity) for each condition in caudal inferior frontal cortex (BA44), ventral premotor cortex and prefrontal cortex anterior to BA44 in healthy controls.....	100
<b>Figure 7</b> Unilateral activity in left PMd in the healthy control group.....	101

**Page**

<b>Figure 8</b> Brain regions in which activity during observation of an active task correlates with accuracy for the 1-back task for the same condition in healthy participants.....	102
<b>Table 5</b> Brain activity correlating with one-back task accuracy in healthy controls...	103
<b>Figure 9</b> T1 weighted brain scans from the seven stroke patients.....	105
<b>Figure 10</b> SPM {Z}s representing brain activity for observing either goal directed (top) or non-goal directed actions in the patient group.....	106
<b>Table 6</b> Observing a goal directed versus non-goal directed action – patients.....	107
<b>Figure 11</b> Increase in PMv/BA44 activation for goal directed actions in patients..	108
<b>Figure 12</b> Mean percentage accuracy rates during fMRI, while observing pinch, grasp and control movements. ....	109
<b>Table 7</b> Decreased activation in patients compared to controls for active tasks.....	110
<b>Figure 13</b> Decreased activity in the affected hemisphere of patients compared to controls whilst watching goal-directed actions with either hand.....	111
<b>Table 8</b> Decreased activation for observing pinch compared to grasp in patients...	112
<b>Figure 14</b> Brain regions in which the difference in activity for observing grasp compared to pinch (grasp minus pinch) is greater in patients than controls.....	112
<b>Figure 15</b> Perilesional activity seen in region of BA44/PMv (at point of cross-hairs) during observation of all goal-directed actions in patient 7 despite extensive infarct.....	113
<b>Figure 16</b> Single subject, two session design matrix.....	168
<b>Figure 17</b> Design matrix for laterality analysis.....	170

<b>Part 3: Critical Appraisal.....</b>	<b>136</b>
<b>1. Development of the research question.....</b>	<b>137</b>
<b>2. Methodological dilemmas.....</b>	<b>138</b>
2.1 Selecting the objects.....	138
2.2 Selecting the hand movements.....	140
2.3 Selecting the control stimuli.....	142
2.4 Piloting the stimuli.....	143
<b>3. Reflections on the research process.....</b>	<b>144</b>
<b>4. Strengths and limitations of the research.....</b>	<b>146</b>
4.1 Strengths .....	146
4.2 Limitations.....	147
<b>5. Future directions and clinical implications.....</b>	<b>148</b>
<b>6. References.....</b>	<b>151</b>

## Appendices

Appendix A: Copy of ethical approval letter.....	152
Appendix B: Information sheet for healthy volunteers.....	155
Appendix C: Information sheet for patients.....	159
Appendix D: Informed consent form for healthy volunteers.....	163
Appendix E: Informed consent form for patients.....	165
Appendix F: Procedures for Statistical Analysis of fMRI Data.....	167

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## **Part 1: Literature Review**

**Can the observation of another's actions enhance the motor performance of stroke patients? A review of the mirror neuron system literature and its implications for stroke rehabilitation.**



### **Abstract**

Motor impairments are a common consequence of stroke, but some patients may be too impaired to participate in physiotherapy. In primates, single cell recordings have identified neurons that are active during both performance and observation of motor tasks. A similar “mirror neuron system” (MNS) in humans could potentially be utilised to augment physiotherapy in stroke patients. This paper summarises the mechanisms underlying recovery from stroke, before reviewing evidence for the existence of a human MNS. Converging evidence demonstrates that in healthy individuals, observation of another’s actions activates a distributed network of brain areas, and can facilitate motor output. Preliminary findings suggest that action observation may also improve motor performance in stroke patients. Further research is required to determine the mechanisms underlying action observation in stroke patients, in order to establish its potential as a rehabilitation technique.

## **1. Introduction**

Stroke is a major cause of disability in all age groups. Most of the survivors from the 150,000 people that have a stroke in the UK each year are left with functional deficits (The Stroke Association, 2008). Stroke can be caused by a clot blocking the blood supply to the brain (ischaemic stroke), or less commonly, by a haemorrhage. The resulting death of brain tissue, or cerebral infarction, can have devastating consequences. Depending on the location of the infarct, survivors of stroke may suffer from disorders of movement, balance, speech and language, swallowing, sensory functioning, bladder and bowel functioning, vision or sleep. They may also suffer from a range of cognitive impairments, emotional lability, depression, pain and fatigue. Moreover, the emotional, physical and cognitive consequences of stroke often have a significant impact on individuals' relationships and vocations.

Movement impairments are a common consequence of stroke, and often have a large influence on patients' independence, identity and quality of life. The use of physiotherapy to minimise motor impairment is based on the principle that repeated practice of motor tasks can promote change within surviving neural networks. However, some patients may be too impaired to use this approach (Pomeroy et al., 2005). Consequently, there is growing interest in alternative approaches to driving change within motor networks in such patients.

In primates, single cell recordings have identified "mirror neurons", which are active during both the performance of a motor task, as well as during observation of the same task performed by another (Rizzolatti, Fagida, Gallese, & Fogassi, 1996).

Research in humans supports the notion of a similar “mirror neuron system” (MNS), located in a distributed network of brain areas, which is active during the observation or mental rehearsal of movements as well as during movement execution. Furthermore, there is evidence that MNS activation can facilitate motor output to target muscles (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995). Therefore, motor performance can potentially be improved by observing others performing the movement, or by mental rehearsal of the movement. This concept has become popular in the field of sports psychology as a technique for improving the motor performance of athletes (Morris, Spittle, & Watt, 2005).

This paper aims to address the question of whether it may be feasible to use the observation of others’ actions as an adjunct to physiotherapy in stroke rehabilitation. It will begin by summarising the mechanisms underlying normal recovery from stroke, and existing treatments for motor-impaired stroke patients. Following this, converging evidence for the existence of a human mirror system will be reviewed, in order to determine the potential for action observation as a therapy technique for motor-impaired stroke patients.

## **2. Background**

### ***2.1 Neuroplasticity following stroke***

The brain’s ability to adapt in response to changes in the environment is known as neuroplasticity. Throughout our lives our brains are constantly developing as we

learn new skills and gain new experiences (Nudo, Plautz, & Milliken, 1997). The brain develops rapidly during early childhood in response to new learning experiences. This is followed by extensive synaptic pruning, in which weaker synapses are eliminated. Although brain development slows in adulthood, plasticity continues as a result of repeated learning experiences or environmental changes. In Braille readers, for example, the cortical representation for the reading finger is enlarged at the expense of the representation of other fingers, due to increased use (Pascual-Leone et al., 1993). Cerebral reorganisation in the somatosensory cortex can occur following limb amputation, resulting in the experience of sensations in the amputated limb when adjacent somatosensory representation areas are stimulated (Halligan, Marshall, & Wade, 1994).

Following acquired brain injury, the brain naturally adapts in order to aid recovery and compensate for functional deficits. Evidence from animal models suggests that the damaged brain is more plastic than the healthy adult brain (e.g., Bury & Jones, 2002). Most natural recovery usually occurs within 6 months of the stroke (Fregni & Pascual-Leone, 2006). According to Hallett (2001), spontaneous recovery following stroke is likely to be due to resolution of oedema (accumulation of fluid), and recovery of function in ischaemic tissues that were not destroyed. In the months following this acute period, recovery is brought about by brain plasticity. Mechanisms for plasticity include strengthening and weakening of synapses, changes in neuronal membrane excitability, and the formation of new synapses (Hallett, 2001).

Behaviours are controlled by complex brain systems involving inhibitory and excitatory inter-connections between multiple regions, and recovery following brain injury reflects the co-ordinated workings of the entire brain to maintain function (Fregni & Pascual-Leone, 2006). Numerous areas are involved in the motor system including the primary motor area (M1), premotor area (PMA), supplementary motor area (SMA), somatosensory areas, dorsolateral prefrontal cortex, the basal ganglia and the cerebellum. Motor recovery following stroke therefore involves changes in activity across this entire network to preserve function.

Early after stroke, motor cortical areas are recruited to a greater extent than at later stages of recovery, and poorer functional outcomes are associated with greater activation of primary and secondary motor areas (Ward, Brown, Thompson, & Frackowiak, 2003; Ward et al., 2006). This may reflect variations in the neural mechanisms that facilitate recovery according to the time since stroke, such as cortical hyperexcitability in the acute stages. Furthermore, after stroke there is a shift in brain activation during affected limb movement from mainly contralateral primary sensorimotor cortex to a more bilateral pattern involving secondary motor areas. This shift is greater in patients with more impairment and in those with greater damage to the corticospinal tract in an attempt to generate motor output to the spinal cord. This mechanism of cortical reorganisation can only be partially successful in recovering motor function, as secondary motor regions are less efficient than primary regions in generating motor output (Ward et al., 2006).

There is evidence of an increase in excitability of the unaffected hemisphere following stroke, suggesting that recruitment of ipsilateral pathways may be

important in recovery (Strens, Fogelson, Shanahan, Rothwell, & Brown, 2003). This may be in part due to reduced use of the paretic hand, but it may also act as a mechanism to minimise damage in the affected hemisphere (Fregni & Pascual-Leone, 2006). However, increased activation in the damaged hemisphere, contralateral to the functional impairment, is associated with better recovery (Hallet, 2001; Fregni & Pascual-Leone, 2006).

Several factors may affect neuroplasticity and functional recovery following stroke. Timing is particularly important – there is a higher chance of inducing behavioural change in acute patients than chronic patients, possibly due to increased neural plasticity early following brain damage. Other factors include the size and location of the infarct (subcortical strokes or those affecting large areas of the motor cortex resulting in poorer prognosis), degree of motor weakness at the time of the stroke (mild impairment associated with better prognosis), cognitive parameters such as motivation, concentration and attention, and age-related differences in capacity for brain reorganisation (Fregni & Pascual-Leone, 2006; Ward & Frackowiak, 2006). Therefore, it is important to establish the mechanisms of recovery in different subgroups of patients, in order to develop appropriately targeted treatments (Ward & Frackowiak, 2006).

## ***2.2 Treatment of stroke patients with upper limb motor impairments***

Rehabilitation of upper limb motor deficits often relies on compensatory strategies – for example, learning to use the unimpaired, non-dominant hand to write, rather than

important in recovery (Strens, Fogelson, Shanahan, Rothwell, & Brown, 2003). This may be in part due to reduced use of the paretic hand, but it may also act as a mechanism to minimise damage in the affected hemisphere (Fregni & Pascual-Leone, 2006). However, increased activation in the damaged hemisphere, contralateral to the functional impairment, is associated with better recovery (Hallet, 2001; Fregni & Pascual-Leone, 2006).

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## ***2.2 Treatment of stroke patients with upper limb motor impairments***

Rehabilitation of upper limb motor deficits often relies on compensatory strategies – for example, learning to use the unimpaired, non-dominant hand to write, rather than

focussing on treating the impaired hand. This is partly due to the need to consider efficiency of time and resources in restoring functional outcomes (Buccino, Solodkin, & Small, 2006). However, this approach can result in the motor representational area for the impaired limb becoming further limited by its disuse (Hallet, 2001).

According to Hluštík and Mayer (2006), rehabilitation should involve the earliest and most intensive possible sensory and motor training of the hand in order to promote plasticity of the affected cortical areas. Although physiotherapy is commonly used, the only specific rehabilitation technique to have undergone a randomised clinical trial (RCT) to date is constraint-induced movement therapy. This approach was developed by Taub, Uswatte and Pidikiti (1999), and involves constraining the unimpaired limb in order to force use of the paretic limb. Constraint induced movement therapy is based on the principle that repeated practice of motor tasks using the paretic hand can promote activity-driven change and recovery within surviving neural networks, as well as strengthening the affected muscles (see Sunderland & Tuke, 2005, for a review). The RCT showed significant improvements in motor functioning of the impaired arm in the constraint induced movement therapy patients compared to the control group (Wolf et al., 2006).

Unfortunately, some patients may be too impaired to use this approach (Pomeroy et al., 2005). Consequently, there is growing interest in alternative approaches to driving change within motor networks in such patients, including pharmacologic interventions, non-invasive cortical stimulation, and somatosensory input (Floel & Cohen, 2006). An exciting new possibility is based on the discovery of mirror



neurons, and the finding that that observation of movements can enhance motor output in healthy individuals. This has led to the suggestion that action observation may improve motor recovery after stroke (Buccino et al., 2006; Pomeroy et al., 2005).

### ***2.3 The mirror neuron system – an alternative approach to rehabilitation?***

In the 1990s, research involving the mapping of neuronal activity in primate motor areas led to the accidental discovery of an unusual population of neurons. Single cell recordings revealed a set of neurons in the ventral premotor cortex (area F5) that discharge during the monkey's performance of hand or mouth object-related actions, as well as during observation of the same tasks performed by another monkey or the experimenter (Rizzolatti et al., 1996). These neurons have been dubbed "mirror neurons", as the observed action seems to be reflected in the motor system of the observer. The same neurons have since been shown to be active when the goal of the action is implied but not visualized, suggesting a role in action recognition and understanding the meaning of actions (Umiltà et al., 2001). A second population of neurons with similar mirror properties has been discovered in the inferior parietal lobule, which connects to area F5 (Fogassi et al., 2005). Additionally, auditory mirror neurons which fire in response to the sounds of hand or mouth actions, as well as during execution of these actions, have been found in primates (Kohler et al., 2002).

Studies in humans have shown “mirror” activation during action observation in Broca’s area, which is located in the left inferior frontal gyrus and traditionally associated with language processing. Broca’s area is often considered the homologue of primate area F5, although there is some ambiguity regarding this homology (Amunts et al., 1999). Human mirror activation has also been seen in the adjacent ventral premotor area (PMv) or Brodmann’s Area (BA) 44, as well as rostral parts of the inferior parietal lobule and secondary motor regions such as dorsal premotor cortex (e.g., Buccino et al., 2001). A similar network has been shown to be active during motor imagery, defined by Jeannerod (1995) as “mental rehearsal of simple or complex motor acts that is not accompanied by overt body movements” (Decety et al., 1994; Galdo-Alvarez & Carrillo-de-la-Pena, 2004; Grafton, Arbib, Fadiga, & Rizzolatti, 1996; Lacourse, Orr, Cramer, & Cohen, 2005). As the inferior frontal gyrus is involved in action observation, mental imagery and action execution, it has been suggested that this area may be involved in forming internalised representations of actions (Jeannerod, 2006).

The involvement of Broca’s area, and the possible role of mirror neurons in understanding others’ actions and intentions, has been extended by a number of authors to potentially explain broader aspects of social cognition, communication and language evolution. For example, Gazzola, Aziz-Zadeh and Keysers (2006) provided evidence for the existence of a human auditory mirror system which is activated to a greater extent by individuals who scored higher on an empathy scale. It has also been proposed that MNS impairment may contribute to poor imitation performance and social abilities in autism. However, research involving imitation and gesture

recognition tasks, which are assumed to rely on the MNS, has provided evidence against a global MNS deficit in autistic children (Hamilton, Brindley, & Frith, 2006).

Stroke rehabilitation is a clinical area in which a human mirror system may prove to be of significance. Increasing activation in “mirror” motor areas, by observing another’s actions or using motor imagery, may promote plasticity in surviving neural networks and improve motor functioning. Primate area F5, where mirror neurons have been located, has a direct role in modulating motor output from the primary motor cortex (M1). Similarly, recordings from hand and arm muscles in healthy human individuals indicate greater excitability in M1 during the observation of movement involving these muscles (Fadiga et al., 1995). There is also evidence that mental rehearsal of movements can improve motor performance (e.g., Fontani et al., 2007). Thus in stroke patients with impaired upper limb function, observation of another’s hand/arm movements, or motor imagery, could potentially facilitate motor output. If this approach is effective, it may be particularly beneficial for those patients who are too impaired to participate in physiotherapy.

The remainder of this paper reviews the evidence for the existence of a human mirror system in healthy individuals and stroke patients, in order to establish whether it may be feasible to apply this notion to the rehabilitation of motor-impaired stroke patients. Although there is a large body of evidence to suggest that motor imagery may improve motor performance, this review focuses on action observation, which is less subjective, and more easily controlled and measured experimentally (Holmes, 2007).

### **3. Review of converging evidence for the existence of a human mirror system, and its implications for stroke rehabilitation**

#### ***3.1 Methods***

Electronic databases (PubMed, Web of Science and PsychInfo) were searched using the terms “mirror neuron system” and “action observation”. References cited within relevant papers were also examined. The review focuses on primary research papers published since 2000, but key references published prior to this have also been included. I have reviewed studies that have used varying methodologies, in order to examine converging evidence for the existence of the MNS and its application in stroke rehabilitation. Studies with relevance to motor facilitation in healthy individuals and stroke patients are included, but papers with a focus on the application of the MNS to social cognition have been excluded.

#### ***3.2 Transcranial magnetic stimulation (TMS) studies***

Transcranial magnetic stimulation (TMS) is a non-invasive method, which can be used to investigate the functions of brain areas by temporarily disrupting their functioning. During action observation, the magnitude of motor-evoked potentials (MEP) from hand muscles can be measured while applying TMS to the primary motor cortex (M1). An increase in MEP implies increased motor output from M1 to the muscles (i.e., increased corticospinal activity).

Fadiga and colleagues provided the first evidence for the existence of a human MNS (Fadiga et al., 1995). They delivered TMS while participants observed an experimenter grasping objects, the objects alone, the experimenter tracing geometric figures in the air with his arm, or detection of the dimming of a light. MEPs were recorded from hand muscles during these four conditions. In the conditions involving the observation of movements, there was an increase in MEP amplitude from the muscles specifically associated with performing the action. This was confirmed by measuring MEPs from the hand muscles of the same participants during execution of these movements. This study therefore provided evidence for a mirror system that is active during the observation of another's movements, and which produces increased motor output to the muscles involved in actually executing the observed actions. This muscle-specific facilitatory effect was later replicated in a TMS study involving observation of an experimenter hand-writing and observation of arm movements (Strafella & Paus, 2000).

Further evidence for a mirror system that matches observed and executed actions comes from a study investigating the temporal patterns associated with observed movements. Gangitano, Mottron and Pascual-Leone (2001) applied TMS to M1 while participants passively observed video clips of an actor grasping an object. This not only resulted in an increase in MEP amplitude in the observer's hand muscles, but the temporal pattern of MEP modulation resembled the time course of the observed action, as if the observers were physically executing the action. This therefore provides support to the notion that observed actions are internalised and represented as one's own. Similarly, another TMS study of action observation has demonstrated that MEPs are greater during observation of a contralateral hand action

than during ipsilateral hand movements, when applying a pulse to M1 (Aziz-Zadeh, Maeda, Zaidel, Mazziotta, & Iacoboni, 2002). As action execution also involves the hemisphere contralateral to the moving body part, this provides additional evidence for a mirror system that matches observed and executed action representations.

Stefan et al. (2005) demonstrated using TMS that action observation can also change long-lasting motor representations. Participants were asked to observe an actor's thumb movements on a computer screen for extended periods. These thumb movements were in the opposite direction to participants' habitual TMS-evoked thumb movement directions, which had been recorded at baseline. Subjects were given a counting task in order to maintain attention to the observed movements. A physical practice task, in which participants practiced performing thumb movements in the direction opposite to the baseline, acted as a within-subjects comparison condition which took place during a separate testing session. Following the observation and physical practice conditions, TMS-evoked thumb movements were measured again. In both conditions, although to a lesser extent in the observation condition, TMS-evoked thumb movements altered in favour of the direction opposite to the baseline condition. This indicates that the human MNS may be important in motor memory formation and motor learning, highlighting its potential for stroke rehabilitation. However, a similar study by the same group (Celnik et al., 2006), showed that in older adults, thumb movement directions only altered following a combination of action observation and physical practice, but not following either condition alone. This may have implications for the application of action observation as a tool in the rehabilitation of older stroke patients.

Most action observation studies have used video stimuli showing an actor's hand, which enables comparison of participants' own movements with those of the actor. However, this methodology cannot control for the visual, social and physiological differences between experiencing one's own action and viewing another's. Therefore, differences between action observation and self-produced actions could potentially arise from a number of factors. Schütz-Bosbach, Mancini, Aglioti and Haggard (2006) addressed this difficulty in a TMS study using the well-established "rubber hand illusion". When participants watch a rubber hand being stroked while they feel synchronous stroking of their own unseen hand, they feel that the rubber hand becomes part of their own body. Asynchronous stroking has no effect, however (Tsakiris and Haggard, 2005). By using a version of this task to manipulate whether an experimenter's hand was experienced as each participant's own hand or as belonging to another, the differences between observing actions attributed to self and other were controlled for. MEPs evoked by motor cortical TMS during both conditions were measured.

In confirmation of previous studies, it was found that action observation facilitated participants' own motor systems when the moving hand was attributed to another, and the effect was specific to the muscle involved in the observed action. However, when the moving hand was attributed to the self, this effect was suppressed. This suggests that the mirror system is agent specific – i.e., the matching of observed actions onto one's own representations depends on whether the observed actions are attributed to oneself or another. The finding that motor responses are suppressed during observation of self-attributed actions has been interpreted as a functional response that prevents inappropriate perseveration (Schütz-Bosbach et al., 2006).

Interestingly, this corresponds with findings that sensory responses resulting from one's own actions are attenuated (for example, when tickling oneself). This mechanism allows us to distinguish between actions originating from self and other, and may be disrupted in schizophrenia (Blakemore, Wolpert, & Frith, 1998; Shergill, Samson, Bays, Frith, & Wolpert, 2005).

In summary, TMS studies have provided neurophysiological evidence to support the existence of a human MNS. They have demonstrated that hand muscles are physiologically involved in the observation of hand actions produced by others. This implies that action observation results in an increase in the excitability of the corticospinal pathway, possibly due to the motor system simulating the observed action under-threshold (Fadiga, Craighero, & Olivier, 2005). Fadiga et al. (2005) have noted that these TMS studies cannot tell us whether the increased activity originates in M1 or in premotor areas connecting to M1, and that brain imaging studies are necessary to establish this. However, if the corticospinal pathway is similarly activated during action observation in stroke patients, this may prove to be a useful rehabilitation tool.

### ***3.3 Respiration rates***

Further physiological evidence of motor facilitation during action observation has been provided by a study investigating respiration and heart rates during observation of another's actions (Paccalin & Jeannerod, 2000). Eleven healthy male volunteers were asked to passively observe an actor performing effortful actions, such as



running on a treadmill at varying speeds or lifting weights. Interestingly, participants' respiration rates increased as the running speed of the actor increased. This suggests there may have been an increase in excitability of the participant's motor systems, facilitated by the observed actions. However, there were no changes in respiration rate when observing weight-lifting, and no changes in heart rate were detected while watching either activity.

It is unclear why there were no changes in heart rate, although it is possible that respiration rates are more sensitive to motor facilitation via action observation. The authors suggested that the absence of a respiration effect in the weight-lifting condition, and also the increase in respiration rate for increased running speeds, may be an effect of the frequency of the actors' movements. The frequency of movement was higher during running than weight-lifting, and particularly during faster running speeds, which may explain the increased respiration rate. It is also likely that the actor's respiration rate was higher for running than for weight-lifting, which would further support the notion that the observers internalised the motor representations that they observed. It would be interesting to examine whether less fit observers had higher respiration rates during observation of effortful actions, as would be expected during performance of the same actions, since this would provide additional support for the notion of internalising the observed movement representations. The authors concluded that central mechanisms related to action performance are activated during the observation of effortful actions, and that this could represent a basis for understanding and imitating others' actions (Paccalin & Jeannerod, 2000).

### ***3.4 Behavioural studies***

Behavioural observations have provided indirect evidence of a human mirror system and the functional outcomes of mirror activity. Everyday experiences of motor learning by observation may reflect a mirror neuron mechanism. In infancy and throughout our lives, we observe and imitate others' actions in order to learn new motor skills and develop existing ones (Lepage & Théoret, 2007). According to Jeannerod (2006), observing the actions of another person creates in the observer a representation of the action, which subsequently facilitates the execution of the action by the observer. It has been suggested that this motor facilitation effect may explain why we tend to mirror one another's movements in social situations without conscious awareness (de Vries & Mulder, 2007).

In a formal study of motor learning by observation, participants learned a finger tapping sequence as efficiently by observation of an experimenter as they did by physically practicing the task (Heyes & Foster, 2002). In sports psychology, action observation has received less attention than motor imagery. However, there is evidence that performance of athletic tasks can be improved by the observation of others. For example, Ram, Riggs, Skaling, Landers and McCullagh (2007) demonstrated that modelling of weight-lifting and balance tasks resulted in improved performance in novice learners compared to imagery and control (rest) conditions.

Behavioural studies can also provide evidence for a mirror system by demonstrating motor interference – i.e., an interaction between an observed action and an executed one (Shmuelof and Zohary, 2007). In motor interference studies, subjects are

required to perform a movement whilst watching an action that is either congruent or incongruent to the one they are performing. Performance is less accurate during the observation of incongruent actions, even when these are irrelevant for the task (Brass, Bekkering, Wohlschläge and Prinz, 2000; Craighero, Bello, Fadiga, & Rizzolatti, 2002). This suggests that observation of congruent actions facilitates the observer's motor system by activation of cortical motor areas.

It is possible that observing incongruent actions reduces performance accuracy due to the increased task complexity. However, observing a robotic arm rather than a human arm does not produce the same interference effect, suggesting the effect is not due to increased task complexity (Kilner, Paulignan, & Blakemore, 2003). Kilner and colleagues suggested that the interference effect is instead a result of simultaneous activation of overlapping neural networks that process human movement observation and execution, therefore providing evidence of a human MNS that is specific to biological motion. This corresponds with earlier findings in primates (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992). However, it is possible that the findings are due to increased familiarity with human hand motion, and that equal familiarity with robot hands would lead to an equivalent interference effect. Kilner et al. (2003) have also noted that controlling biological motion is problematic, and further studies will be required to assess exactly which aspect of the observed movement triggers interference.

### ***3.5 Clinical neuropsychological evidence***

Examining the effects of brain injury on behaviour can provide important information regarding the functions of the affected brain areas. Buxbaum, Kyle and Menon (2005) investigated the relationship between recognition and execution of object-related (transitive) actions in stroke patients with ideomotor apraxia, which results from damage to parietal areas. Patients with ideomotor apraxia are impaired in pantomiming gestures using familiar objects, despite understanding how the objects should be used and having the physical ability to perform the required movements. This implies a deficit in the ability to form representations of transitive actions (Jeannerod, 2006).

In a test of action recognition, Buxbaum and colleagues asked left-hemisphere stroke patients, with or without ideomotor apraxia, to select which of two videotaped gestures correctly matched an object-related verb (e.g. “hammering”). After controlling for language comprehension, apraxic patients showed significantly higher error-rates than non-apraxic patients, particularly when the incorrect video showed errors in hand posture (such as splayed fingers for a hammering gesture). These findings were interpreted as evidence for impaired recognition of hand postures during transitive actions in patients with ideomotor apraxia. Importantly, regression analysis revealed that scores on this gesture recognition task were strongly predicted by scores on a gesture imitation task. The authors noted that this may imply the presence of a mechanism that underlies both recognition and imitation of complex, skilled, object-related movements.

Lesion analysis indicated that deficits in the recognition and imitation of transitive gestures, and particularly hand posture, were associated with the left inferior parietal lobe and intraparietal sulcus. Lesions of Broca's area were not significantly associated with deficits in recognition and imitation, suggesting greater importance of the inferior parietal lobe in recognition and imitation of complex familiar actions and hand postures. Buxbaum and colleagues concluded that skilled gesture representations, evoked during production and recognition of pantomimed object-related actions, are located in the left inferior parietal lobe and intraparietal sulcus, and that they encode hand postures that are specific to the functional use of particular objects (Buxbaum et al., 2005). This study therefore supports the notion of a human mirror neuron system that applies to complex transitive movements, as well as the simple grasping and finger movements shown in previous studies. It also demonstrates the importance of parietal areas in both recognition and execution of object-related actions.

### ***3.6 Electroencephalography (EEG) studies***

Electroencephalography (EEG) involves the measurement of electrical activity produced by the brain, usually recorded from electrodes placed on the scalp. It enables a direct measure of brain activity and has a high temporal resolution, although it is limited by its spatial resolution. In intracranial EEG, activity is recorded from the surface of the brain rather than the scalp, enabling a higher spatial resolution than surface EEG.

In an intracranial EEG case study of a 19-year-old male epilepsy patient, Tremblay et al. (2004) recorded activity from surgically implanted subdural electrodes over the patient's left frontotemporal cortex. Experimental stimuli included an image of a static hand, video clips of the same hand with the index or middle finger moving vertically, and a blank screen. The patient was instructed to either passively observe the stimuli or to move his middle or index finger, and EEG signals were recorded from the subdural electrodes. Similar changes in brain activity (a reduction in alpha band activity) were seen both during action observation and action execution, in Broca's area and M1. This study therefore provided direct evidence that these brain areas are involved both in observation and execution of simple finger movements. However, the study is limited due to the nature of the methodology, which enabled inclusion of only a single participant with neurological complaints. Caution is therefore needed when generalising these findings to healthy individuals.

In contrast to the behavioural study by Kilner et al. (2003), a recent EEG study revealed that robotic as well as human actions may activate the human mirror neuron system, as indicated by mu wave suppression during action observation (Oberman, McCleery, Ramachandran, & Pineda, 2007). Furthermore, there was no difference in magnitude of mirror activity when the observed robotic hand grasped an object, compared to when no object was present. The discrepancy between this finding and the behavioural study by Kilner et al. (2003), as well as evidence in primates that the MNS is specifically selective for biological, goal-directed actions (di Pellegrino et al., 1992), may be accounted for by varying methodologies. Oberman et al. (2007) have suggested that if the MNS can be activated by robotic actions, this may reflect evolutionary differences in the primate and human mirror systems. The authors

comment that as the spatial resolution of EEG is low, it is difficult to locate the exact location of brain activity producing these results, and that other techniques such as functional magnetic resonance imaging (fMRI) may have greater power to identify differential effects of properties such as volition and presence of an object on the modulation of the MNS.

### ***3.7 Brain imaging studies***

Functional imaging studies allow the non-invasive assessment of distributed regional brain activation during the performance of specified tasks. They have provided convincing evidence for the existence of a human MNS and its properties. Early imaging studies using positron emission tomography (PET) indicated that observation of grasping actions activated left inferior frontal gyrus, left inferior parietal cortex, left supplementary motor area, right dorsal premotor cortex, middle temporal gyrus, and the superior temporal sulcus (Grafton et al., 1996; Rizzolatti, Fadiga, Matelli, et al., 1996). This brain activation during action observation partly overlapped with the activation seen when subjects executed the same actions (Rizzolatti, Fadiga, Matelli, et al., 1996). More recent neuroimaging studies have used functional magnetic resonance imaging (fMRI), which has a higher spatial and temporal resolution than PET.

In an fMRI study, Buccino et al. (2001) showed that mirror activation is not limited to the observation of hand movements. Participants were shown static images or video clips of object manipulation by the hand, foot and mouth, or the same actions

mimicked by these effectors in the absence of the object. Interestingly, the active fronto-parietal regions during action observation corresponded somatotopically to the effectors viewed in the stimuli. Although no action execution condition was included in the study, the topographic organisation of activation during hand, foot and mouth observation corresponded to the classical motor organisation of the premotor cortex demonstrated by Penfield and Rasmussen (1952). Activation of Broca's area and premotor regions was greater during the video clips with and without objects, than during static images of the actions, suggesting that the MNS is activated to a greater extent by moving images. Additionally, parietal areas were active during video clips that involved objects and not those without an object. This parietal activation was also somatotopically organised, and depended on the effector used, as would be expected during object manipulation. It was concluded that observed actions are mapped onto the corresponding motor representations of the frontal lobe and, and also in the parietal lobe in the case of object-related actions. This study therefore revealed the neural substrates of a human matching mechanism that maps the observed actions onto the observer's somatotopically organised motor representations.

Grèzes, Armony, Rowe, and Passingham (2003) used fMRI to examine the effects of action observation versus execution, with and without the presence of an object. They found that observation and execution of grasping actions were commonly associated with a distributed network of areas, and confirmed the importance of the ventral premotor cortex (PMv) and Brodmann Areas (BA) 44/45 in the inferior frontal gyrus as integral parts of the human mirror system. Other mirror areas included the intraparietal sulcus, dorsal premotor cortex and superior temporal sulcus



bilaterally, and the right parietal operculum. This network of areas was active regardless of whether the observed grasping action was directed towards an object. Together with the fMRI study by Buccino et al. (2001) and the EEG study by Oberman et al. (2007), this suggests that the human mirror system may be more flexible than the monkey's, which seems to respond to only object-related actions.

An fMRI study by Johnson-Frey et al. (2003) investigated the effects of varying the hand-object interaction style and type of object during action observation. Participants performed a 1-back task (judging whether any consecutive stimuli were repeated) in order to maintain attention to the stimuli. Experimental stimuli consisted of static images of a hand grasping or simply touching familiar objects such as bottles, or unfamiliar three dimensional shapes. In confirmation of previous findings, bilateral precentral and inferior frontal gyri were selectively activated in response to hand-object interactions. Activation was greater when viewing images of grasping objects than images of touching objects, regardless of the type of grasp and the familiarity of the object. Additionally, despite the earlier finding that static images activate mirror areas to a lesser extent than moving images (Buccino et al., 2001), this study demonstrated that static images are sufficient to activate the mirror system.

Further investigation of the context in which objects are grasped revealed that premotor mirror neuron areas may be involved in understanding others' intentions (Iacoboni et al., 2005). In this fMRI study, video clips showed a hand grasping a mug in a "drinking" context, or in a "cleaning" context. Both contexts showed the same set of objects, but in the cleaning context the objects were arranged as if the model in the clips had just had tea (e.g., jam jar lid off, mug empty, and milk jar tipped over).

When the hand grasped the cup in the drinking condition, there was greater activation in the right inferior frontal cortex than in the cleaning condition. This difference was not found in control stimuli showing the same two contexts, without the presence of a hand. The authors interpreted this finding as evidence that the right inferior frontal gyrus is important as a mirror area, and also in encoding intentions. However, it is possible that this difference between contexts may have been associated with a more basic difference in stimuli between the two conditions. For example, the presence of liquid in the mug in the drinking condition would have made the mug heavier than in the cleaning condition, therefore requiring increased effort and differential biomechanical qualities.

If action observation is to be used as a therapy for stroke patients, imitation may be an important aspect of the therapy. Buccino and colleagues (Buccino, Vogt et al., 2004) demonstrated that brain regions involved in action observation are also involved in imitation learning of guitar chords. These areas included the inferior parietal lobule, the posterior part of the inferior frontal gyrus, and the adjacent premotor cortex. The middle frontal gyrus (BA 46) appeared to be particularly important in action observation and imitation learning. They concluded that new motor learning involves the MNS, and in particular BA 46. This may have implications for the ability of stroke patients to relearn motor skills via imitation if they have damage to this area. Behavioural data from this study was not fully reported, and although two subjects were excluded for poor quality of imitation, it is unclear whether the remaining participants accurately imitated the guitar chords on all trials. Therefore, the increased brain activation was associated with intent to imitate, but not necessarily with accurate reproduction of the observed movements.

Shmeulof and Zohary (2006) used fMRI and single-subject region of interest analysis to confirm that the anterior intraparietal cortex forms part of the human mirror system. Fourteen healthy participants observed video clips of a hand grasping everyday objects, and also performed simple movements. The anterior intraparietal cortex was active during observation and execution of hand actions. Furthermore, this area was activated to a greater extent by observation of movements of the contralateral hand than movements of the ipsilateral hand. This supports the notion that action observation evokes an internal simulation of the movement using the observer's own motor system (Shmeulof and Zohary, 2006; 2007), and confirms the TMS findings of Aziz-Zadeh et al. (2002).

In a recent fMRI study, healthy participants observed static photographs of human models and were required to predict whether the models could reach a target placed in front of them (Lamm, Fischer & Decety, 2007). The network of areas recruited by subjects during this task included primary and secondary somatosensory cortices, inferior and superior parietal areas, and right ventral premotor cortex, which have been previously established as mirror areas. Again, this suggests that mirror neurons might provide a mapping from visual perception onto action simulation, a process that allows us to predict the actions of others. This study replicates previous findings regarding the neural correlates of the MNS, and additionally provides support for theories regarding the role of the MNS in understanding and predicting others' actions.

An important property of the mirror system highlighted by fMRI research is that mirror areas are activated to a greater extent by movements within the observer's motor repertoire. For example, in a study in which humans observed the facial actions of humans, dogs and monkeys, only actions within the human repertoire, such as biting, activated the premotor cortex. Non-human facial actions such as barking, however, only activated visual areas (Buccino, Lui et al., 2004). Studies of dancers have extended this finding to the observation of complex human movements. Calvo-Merino, Glaser, Grèzes, Passingham and Haggard (2005) compared brain activity using fMRI in experts in classical ballet, capoeira dancers, and inexpert control subjects. Activation in mirror areas, including bilateral premotor cortex and intraparietal sulcus, right superior parietal lobe and left posterior superior temporal sulcus, was greater when viewing movements within participants' own motor repertoires.

A study of male and female ballet dancers by the same group investigated whether this effect was due to increased visual familiarity rather than increased motor familiarity with the movements. Participants in this study were male and female ballet dancers, so that visual familiarity with the stimuli (classical ballet movements) remained constant across subjects, but motor familiarity could be manipulated by selecting gender-specific movements as experimental stimuli. Premotor, parietal and cerebellar areas were activated to a greater extent when the observed ballet movements were within the participants' own movement repertoire (same-gender movements), compared with opposite-gender movements that were frequently seen but not performed. As well as providing new evidence that the cerebellum forms part of the action observation network, these results confirmed that actions are understood

not only by visual recognition, but also by the activation of motoric representations within one's own repertoire (Calvo-Merino, Grèzes, Glaser, Passingham, & Haggard, 2006).

These findings have been replicated in another fMRI study of dancers using a within-subjects design. Participants were scanned weekly over a period of five weeks while learning a movement sequence. Increasing familiarity and self-rated ability to perform the sequence was associated with increased activation in the action resonance network whilst viewing videos of the movement sequence (Cross, Hamilton, & Grafton, 2006). This suggests that when a movement becomes part of an individual's motor repertoire, there is increased resonance in the mirror system during passive observation of the movement. As well as demonstrating that the human MNS can be activated by complex, intransitive actions, these studies of dancers have given further support to the notion that humans understand others' actions by motor simulation. This may have important implications for stroke rehabilitation if the observed movements are no longer part of the patients' repertoires, as patients may not be able to form motor representations of the observed actions.

Gazzola and colleagues (2007) studied two aplasic individuals who were born without arms or hands. They aimed to investigate whether an action resonance system exists in the brain during observation of hand actions, even when the individual has never been able to perform those actions and therefore do not have motor programmes that can resonate with them. Interestingly, the two aplasic subjects showed brain activation in areas corresponding to the effectors that they

would use to execute the observed actions, such as the feet or mouth. They concluded that the brain has a capacity to mirror actions that deviate from the embodiment of the observer, by recruiting areas involved in the achievement of the observed goals for that individual. This raises the possibility that in stroke patients who have lost the ability to use a hand, action observation may recruit alternative brain areas such as hand areas contralateral to the observed hand.

As Shmuelof and Zohary (2007) have commented, a difficulty with fMRI is its coarse spatial resolution. It is possible that there are various different neuronal populations within any given voxel, some of which are specific to visual aspects and others which are specific to motor elements. Repetition suppression, or fMRI adaptation paradigms, can address this difficulty (Grill-Spector et al., 1999). If the same neurons are firing repeatedly, for example when watching the same stimulus repeatedly, a reduction in activation is seen (i.e., the brain region shows adaptation). Therefore, if the same neurons fire both during observation and execution of a movement, adaptation should be seen not only during within-modal adaptation (repeated observation of a stimulus, or repeated execution of an action), but also when observation is followed by execution and vice-versa (cross-modal adaptation).

Dinstein, Hasson, Rubin and Heeger (2007) have used repetition suppression in an action observation and execution study. Healthy participants played a game of rock-paper-scissors against a virtual opponent, without instruction regarding which movements to select on each trial. This task therefore involved both observation (of the opponent's movements) and execution, and sometimes involved repetition of observed or executed movements on consecutive trials. A small number of cortical

areas demonstrated within-modal adaptation for observed movements and executed movements. These areas correspond well with mirror areas identified in previous literature, including PMv and anterior intraparietal sulcus, as well as other frontal, parietal and occipital areas. However, no cross-modal adaptation was found which would have provided more convincing evidence for the existence of mirror neurons that fire both during action observation and execution. The authors suggest that their protocol was not suitable for capturing the cross-modal adaptation effect, due to the fact that different forms of neural adaptation can take place at different timescales, from a few hundred milliseconds to hours and days.

Repetition suppression has also been used to isolate different levels of processing within the action observation system, and has demonstrated that different brain regions show adaptation for different aspects of observed goal-directed movements (Grafton & Hamilton, 2007). For example, kinematic features such as arm trajectory and grip configuration engage visual association areas, while the anterior intraparietal sulcus seems to be involved in representing the identity and function of the grasped object. This shows that the mirror system is organised in a hierarchy of brain regions, much like the visual system.

In summary, neuroimaging studies have provided convincing evidence for the existence of a human MNS and its neural correlates, and methodological developments such as repetition suppression have provided further insight into its properties. Parts of the inferior frontal cortex, in particular Broca's area and PMv, seem to be important in action observation and execution. These areas have been considered the putative homologue of area F5, where primate mirror neurons have

been located, although care should be taken when comparing between monkey and human data due to differing methodologies and difficulties in establishing anatomical homologies (Grafton & Hamilton, 2007). It seems that the human mirror system also comprises rostral parts of the inferior parietal lobule and secondary motor regions such as dorsal premotor cortex. Some studies have found evidence that additional brain areas form part of a more extensive mirror network, although caution is needed when designing and analyzing fMRI experiments to avoid overestimation of the extent of this network. Resonance with the observed movements seems to be a particularly important aspect of MNS activation, and actions that are within one's own repertoire activate the MNS to a greater extent than those that are not.

It is worth noting that the fMRI studies reviewed here have varied in aspects such as the body part moved, whether or not participants are required to execute movements, the nature of the visual stimuli (first person versus third person, presence and type of object, and static versus moving), whether or not an active task was involved (as opposed to passive observation), and the size and characteristics of the participant population. Furthermore, paradigms that have required participants to perform actions other than simple grasping and finger movements may be limited by head movement during scanning, which can cause artefacts in the data. Despite this heterogeneity in study design, the inferior frontal cortex (PMv and Broca's area) and inferior parietal areas seem to be consistently activated during observation of actions. This suggests that these are orchestrating regions within the human mirror system.



### ***3.8 Stroke rehabilitation***

Despite the wealth of evidence regarding the MNS, interest in applying this knowledge to stroke rehabilitation has only emerged relatively recently. There has been some evidence that on trained tasks, motor imagery training may improve the motor functioning of stroke patients with motor impairments (de Vries & Mulder, 2007; Dijkerman, Letswaart, Johnston, & MacWalter, 2004; Malouin, Belleville, Richards, Desrosiers & Doyon, 2004; Page, Levine, Sisto, & Johnston, 2001; Sharma, Pomeroy, & Baron, 2006). However, as noted previously, although motor imagery may recruit the same neural mechanisms as action observation, it is more subjective, difficult to measure and harder to control experimentally (Holmes, 2007). Therefore, it is not possible to draw clear conclusions regarding the use of imagery in stroke rehabilitation. Furthermore, there is evidence that in healthy individuals, action observation is more beneficial than imagery in motor learning (Ram et al., 2007). To date, there has been only one completed study of action observation in stroke patients, and a report of preliminary results of an ongoing trial.

The ongoing trial (Buccino et al., 2006) involves action observation and imitation of everyday movements in stroke patients with upper limb motor impairments. Patients up to the age of 70 years with first-ever ischaemic stroke in the territory of the middle cerebral artery, with no cognitive impairments or depression, are included in the study. In addition to traditional neuro-rehabilitation, participants are asked to observe movies, approximately 15 minutes in length, of daily actions such as lifting a cup of coffee. They are then required to perform the observed action using their impaired arm. Initial findings indicate an improvement in motor performance, as evaluated using a range of measures of motor function before, during and after

intervention. However, the number of participants included in the initial analysis, the frequency and time course of the intervention, and statistical significance levels of the results have not yet been reported. It is also unclear if there is a comparison group of patients who are only receiving traditional rehabilitation, and the details of the traditional intervention remain to be clarified.

A completed study by the same research group demonstrated positive effects of “action observation therapy” for stroke patients with moderate, chronic deficit of the upper limb resulting from medial artery infarction (Ertelt et al., 2007). Patients were randomly assigned to experimental or control groups. In the experimental condition, eight stroke patients observed 6-minute video sequences of daily actions of increasing complexity, assisted by a psychologist who kept them attentive. This was followed by 6 minutes of physical practice, in which patients were required to perform the movement shown in the video sequence. In total, each daily treatment session lasted 90 minutes. Patients did not undergo any other treatment during the study. Motor performance was compared with a control group which was matched for age and duration of former therapies. The control group watched videos of geometric symbols and letters instead of goal-directed movement sequences, but participated in physical practice as in the experimental group. Significant improvement was found in the experimental group over the course of a 4-week treatment programme, as compared with their pre-treatment baseline and with the control group. This improvement was maintained eight weeks after the end of the intervention.

Interestingly, an fMRI investigation of the same participants, in which they were required to manipulate everyday objects, revealed a significant increase in activation in a network of motor areas over the course of the treatment. These areas included bilateral PMv, bilateral superior temporal gyrus, the supplementary motor area (SMA) and the contralateral supramarginal gyrus. The authors concluded that action observation therapy reactivates areas of the MNS, in order to improve motor functioning.

These promising early findings show the potential for the use of action observation as a therapy tool. It is, of course, important to consider potential confounding factors. For example, as it is not possible for experimenters and patients to be blinded to treatment groups, motivational factors may have influenced the results. If motivational factors differed between the two groups, there may have also been differences in physical practice between sessions. If so, the observed increase in cortical activation in the experimental group could potentially be due to increased use of the paretic hand, as well as action-observation driven activity. Furthermore, although patients were assisted as an attempt to keep them attentive, it is possible that the control stimuli were less engaging than the experimental stimuli. Differences between groups in working memory, attention and fatigue could also potentially have influenced the results. Although the study excluded patients with depression and severe cognitive impairments (impaired consciousness, severe to moderate aphasia, anosognosia, neglect, amnesia and dementia), no formal measure of cognitive abilities in the two groups was reported.

Despite these considerations, this study has exciting implications for the future of stroke rehabilitation, and the results will need to be replicated in randomised controlled trials using much larger sample sizes. It will be important to establish which aspects of the therapy are most likely to be beneficial. For example, if action observation without imitation is beneficial, this finding would have significant implications for patients who are too impaired to imitate the movements. The frequency and number of repetitions required for optimal results will also need to be investigated, as well as aspects of the observed stimuli such as types of movements and viewing perspective.

Establishing the neural mechanisms underlying action observation in different groups of patients will be particularly important before conducting large scale clinical trials. It is possible that some lesion sites may result in preserved responses to action observation, but in different networks from those seen in healthy individuals. Potential loss of resonance with observed movements as a result of reduced motor repertoire will be an important factor to investigate, and potential age-related changes in MNS activation will also require consideration. Moreover, it is crucial to determine whether activation of the MNS in stroke patients can result in motor output to the paretic limb. Knowledge of these mechanisms in patients with varying lesion sites and at varying stages of recovery will enable researchers to determine which individuals may benefit most from this intervention, in order to plan clinical trials.

#### **4. Conclusions**

There is a large body of converging evidence for the existence of a human mirror system, which is active both during observation and execution of movements. Neuroimaging studies have demonstrated that the MNS comprises a diverse network of brain regions. The inferior frontal cortex (Broca's area / ventral premotor area) and inferior parietal areas seem to be particularly important areas in the human mirror system, in line with evidence from the initial studies of primates. Furthermore, there is substantial evidence to suggest that in healthy individuals, MNS activation leads to facilitation of motor output, thereby potentially improving motor performance by the mere observation of actions.

Research investigating the utilisation of action observation in stroke patients with motor impairments is still in its early stages. However, the existing work has shown promising results. Further studies will be required to replicate this evidence, but before this it will be important to understand the neural correlates of action observation in stroke patients, and also whether action observation can facilitate motor output to the muscles in these patients. We will then be able to conclude if and how action observation may be feasible as a rehabilitation tool to augment physiotherapy. By studying patients at varying stages of recovery and with a range of lesion sizes and locations, we will also learn which patients are most likely to benefit from such a treatment. This will enable future interventions to be targeted towards those who have the potential to gain from them.

In conclusion, the discovery of the mirror neuron system has exciting implications for the rehabilitation of stroke patients with motor impairments. Further research will demonstrate whether it is truly feasible to utilise action observation as a therapeutic tool, which could potentially greatly improve the quality of life of those suffering from stroke.

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## **Part 2: Empirical Paper**

### **Observing Actions After Stroke: Investigating the Potential of the Mirror Neuron System as a Rehabilitation Tool**

## **Abstract**

A brain network known as the “mirror neuron system” (MNS) is active both during the performance and observation of motor tasks, and can facilitate output to muscles. This study investigated whether action observation leads to MNS activation in stroke patients with motor impairments, in order to establish its potential as a rehabilitation tool. Two experiments were carried out using a 3x3 mixed factorial design. Stroke patients, age-matched healthy volunteers and younger healthy volunteers participated in a “one-back task”, in which they observed and responded to video clips of hand movements (pinch, grasp and non-goal directed movements). Eight stroke patients, 11 age-matched controls, and 16 younger controls participated in Experiment 1, in which accuracy rates on the task were recorded. In Experiment 2, eight stroke patients, nine age-matched controls and 11 younger controls performed the one-back task during functional magnetic resonance imaging (fMRI). In the healthy participants, there was a positive correlation between task accuracy and activity in left premotor and inferior parietal cortices, implying the one-back task may be a novel behavioural correlate of MNS activity. Behavioural and fMRI results indicated that MNS activity was present in the stroke patients. However, there was a reduction in MNS activity compared to controls, particularly for the observation of more functionally impaired movements (pinch). No age-effects were found in either experiment. These findings suggest that action observation has potential as an intervention for stroke patients across the adult age range. Its utility as a rehabilitation tool may be dependent on patients’ existing motor abilities, and the clinical implications of this are discussed.

## **1. Introduction**

### ***1.1 The consequences of stroke***

Stroke is a leading cause of death and disability in the UK. For those who survive, the impact can be overwhelming. There are often huge physical, vocational, financial and emotional costs to stroke victims and their carers, as well as a significant impact on National Health Service (NHS) resources. It is therefore important to develop therapeutic interventions that can relieve the distress associated with stroke, and that are also efficient and cost-effective.

Motor impairments are a common consequence of stroke. Damage to any of the diverse brain regions involved in movement can result in weakness of the contralateral side of the body (i.e., hemiparesis). Currently, rehabilitation relies on physiotherapy, based on the principle that repeated practice with the paretic limb can drive plasticity within the surviving neural networks. In turn, this can improve motor output. Unfortunately, this approach cannot be used for those who are too impaired to use their paretic limb (Pomeroy et al., 2005). Consequently, there is growing interest in alternative approaches to driving change within motor networks in such patients.

### ***1.2 The Mirror Neuron System (MNS)***

Psychological techniques such as mental imagery or observation of others' actions may provide a potential solution to this problem (Buccino, Solodkin, & Small, 2006;

Pomeroy et al., 2005). In healthy individuals, mental rehearsal of movements, or passive observation of others' actions, activates a network of brain regions that is also involved in the physical execution of these movements. This network is known as the "mirror neuron system" (MNS) (Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). Importantly, in healthy individuals activation of the MNS also facilitates motor output to muscles (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995), and can enhance motor performance (Brass, Bekkering, Wohlschlage, & Prinz, 2000; Craighero, Bello, Fadiga, & Rizzolatti, 2002; Heyes & Foster, 2002; Ram, Riggs, Skaling, Landers, & McCullagh, 2007). Thus, activation of the MNS by simply observing another's actions, or by producing mental images of these actions, could potentially be used in conjunction with physiotherapy to improve motor functioning in stroke patients.

There are a number of factors that may influence MNS activation. The MNS appears to be particularly active during "goal-directed" actions that involve interactions with objects (Buccino et al., 2001; Johnson-Frey et al., 2003). Another important property of the MNS is that it is activated to a greater extent for actions that are within the observer's own motor repertoire, i.e. movements that they can "resonate" with. For example, studies of dancers have shown that action observation activates the MNS to a greater extent when the movements are within the observer's motor repertoire, compared to movements that the observer has seen but has never performed (Calvo-Merino, Glaser, Grèzes, Passingham, & Haggard, 2005; Calvo-Merino, Grèzes, Glaser, Passingham, & Haggard, 2006; Cross, Hamilton, & Grafton, 2006). The MNS may also be affected by age. A TMS study has shown that action observation in young adult participants can enhance motor learning (Stefan et al., 2005).

However, in a similar study involving a group of older adults, the same effect was only seen when action observation was combined with overt motor training (Celnick et al., 2006). This suggests that age related changes may reduce the effects of action observation as a motor training technique.

Neuroimaging studies have shown that the MNS includes the ventral premotor cortex (PMv) or Brodmann Area (BA) 44, rostral parts of the inferior parietal lobule, and secondary motor regions such as the dorsal premotor cortex (Buccino et al, 2001; Grafton, Arbib, Fadiga, & Rizzolatti 1996; Pomeroy et al., 2005). Left PMv/BA44, or nearby Broca's area in the left inferior frontal gyrus, seems to be a particularly important part of the MNS and has consistently been activated during neuroimaging studies of action observation in humans. This brain region is the putative homologue of primate area F5 (Rizzolatti, Fogassi, & Gallese, 2002), where mirror neurons were originally discovered (Rizzolatti et al., 1996). Area F5 has direct anatomical connections to the primary motor cortex (M1), which communicates with muscles via the spinal cord to drive movement. Thus, the facilitation of motor output seen in human MNS studies may result from activation of PMv/BA44 leading to increased excitability of M1. This would in turn drive motor output to the muscles.

Functional neuroimaging studies have not detected changes in M1 activation during action observation. However, transcranial magnetic stimulation (TMS) studies have shown an increase in motor-evoked potentials from specific hand and arm muscles, during the observation of another's movements that involve the same muscles (Fadiga et al., 1995; Strafella & Paus, 2000). This implies that there is indeed an increase in excitability in M1 (i.e., under-threshold firing of neurons) during the

observation of movement, which produces an increase in corticospinal activity to the specific hand muscles involved in the observed action.

### ***1.3 The MNS as a rehabilitation tool***

These studies suggest that, if the MNS, and in particular PMv/BA44, can be activated in stroke patients with motor impairments, there is potential for it to be used as a rehabilitation technique for individuals who are too impaired to use physiotherapy alone. Previous studies investigating the effects of motor imagery training in stroke patients have shown some improvements in motor functioning following training (de Vries & Mulder, 2007; Dikjerman, Ietswaart, Johnston, & MacWalter, 2004; Malouin, Belleville, Richards, Desrosiers, & Doyon, 2004; Page, Levine, Sisto, & Johnston, 2001; Sharma, Pomeroy, & Baron, 2006). However, motor imagery studies have been criticized due to the fact that imagery is more subjective and harder to measure and control experimentally than action observation (Holmes, 2007).

A recent study has been the first to investigate “action observation therapy” in stroke patients with motor impairments (Ertelt et al., 2007). Eight stroke patients observed videos of everyday actions, and were then asked to imitate these actions. They underwent daily 90 minute treatment sessions for four weeks. Compared to a group of stroke patients who participated in a control task, the patients in the experimental group showed significant improvement on standardised motor functioning tasks, which was maintained eight weeks following the intervention. Furthermore, over the course of the training period, the patients in the experimental group showed

significant increases in activation in a network of motor areas, including PMv, when performing a simple motor manipulation task during functional Magnetic Resonance Imaging (fMRI). The control patients did not show this increase in brain activation. This suggests that the action observation therapy may have stimulated recovery of motor neural networks, enabling an improvement in performance. Buccino et al. (2006) have similarly reported initial findings that action observation appears to be beneficial as a treatment for stroke patients with motor impairments. However, full details of this ongoing study are not yet available.

#### ***1.4 Aims***

These promising findings suggest that action observation may indeed activate mirror areas in order to promote motor recovery in stroke patients. Before planning large-scale trials, however, it is important to have a clear understanding of the mechanisms underlying action observation in stroke patients. Establishing the factors influencing MNS activation in stroke patients will have important implications for how action observation would be delivered as an intervention in clinical settings.

To date, there have been no studies of the direct neural correlates of action observation in stroke patients. Although Ertelt et al. (2007) investigated the extent of brain activation during a motor manipulation task before and after “action observation therapy”, they did not examine the neural basis of the action observation itself. Therefore, the increases in brain activation may have been due to a factor other than action observation, such as increased motivation in the experimental group resulting in increased practice of motor tasks between sessions.

Furthermore, there have been no previous studies of the immediate behavioural effects of action observation in stroke patients. Motor interference studies have provided a behavioural measure of MNS activation in healthy individuals. They have demonstrated that action observation can facilitate or interfere with performance on a motor task, depending on whether the observed action is congruent or incongruent to the performed action (Brass et al., 2000; Craighero et al., 2002). However, to date, there have been no such studies providing an immediate behavioural measure of MNS activation in stroke patients.

The current study therefore aimed to use behavioural and fMRI techniques to directly address the question of whether “mirror” activity is elicited in stroke patients with upper limb motor impairments, when observing another’s hand actions. We intended to characterise the degree and location of MNS activity in stroke patients, and the factors that affect this. The purpose of the study was to determine the potential utility of action observation as a rehabilitation strategy, by establishing the underlying mechanisms in stroke patients. If action observation elicits activity in the MNS of stroke patients, it may also lead to an improvement in motor performance by re-activating the neural networks involved in motor functioning.

### ***1.5 Hypotheses***

A “one-back” task was developed, in which participants were required to detect whether any two consecutive video stimuli of hand actions were identical. This allowed a novel behavioural measure of MNS activity, by recording accuracy rates



on the task. Using an active task which required responses, rather than a passive action observation task, also enabled us to ensure that participants were attending to the stimuli during fMRI. Similar orienting one-back tasks have been used by others for fMRI studies of action observation (Grafton & Hamilton, 2007; Johnson-Frey et al., 2003), but they have not been used as a behavioural measure of MNS activity. We carried out separate fMRI and behavioural studies using this task to enable us to assess performance on the task outside of the scanning environment. We used the one-back paradigm to investigate a number of hypotheses:

1. It was hypothesised that action observation in healthy individuals would activate a network of brain regions, in particular left PMv/BA44, as seen in previous neuroimaging studies.
2. We expected to replicate previous findings that in healthy individuals object-related (“goal directed”) actions activate the MNS to a greater extent than actions that do not involve an object (“non-goal directed”).
3. As the MNS constitutes a number of diverse brain regions, we expected that in stroke patients there would be some residual activity in undamaged parts of the mirror network during action observation. We were particularly interested in the degree of activity evoked in PMv/BA44 in stroke patients during action observation, due to the anatomical connections between this area and the primary motor cortex.
4. We predicted that performance on the one-back task would reflect MNS activation, and therefore that accuracy rates on the task would correlate with brain activity during action observation.

5. Previous studies have shown that MNS activation depends on the observer's motor repertoire (i.e., their "resonance" with the observed action). Therefore, it was hypothesised that MNS activity, as indicated by extent of brain activity and accuracy rates on the one-back task, would be preserved only for actions remaining in each patient's motor repertoire, i.e. actions that they were still able to perform.
6. Finally, most studies of action observation in healthy individuals have been conducted on samples of young participants. However, stroke is relatively rare in the younger age groups, and therefore aging effects may have important implications for utilising action observation as a therapeutic technique. In healthy individuals, there is an age-related decline in motor functioning, and there are associated changes in brain activation (Cabeza, 2001). However, there have been no brain imaging studies of age-related changes in MNS activity. The TMS studies by Stefan et al. (2005) and Celnick et al. (2006) have suggested that there may be age-related decreases in MNS activation during action observation. We therefore hypothesised that we would see age-related decreases in brain activation during action observation, and similar decreases in task accuracy.

## 2. Methods

### 2.1 General Methods

#### *2.1.1 Participants*

Stroke patients were recruited from outpatient and inpatient services at the National Hospital for Neurology and Neurosurgery, Queen Square, London. Patients with receptive language difficulties, severe impairment of attention and working memory, or reports of other severe cognitive difficulties, were excluded from the study. Eight stroke patients (4 male, 4 female) with upper limb motor impairments were recruited for both the behavioural and fMRI study (Table 1). Half the patients had left-sided impairments (i.e., right-sided lesions), and half had right-sided impairments. Their mean age was  $59.9 \pm 7.5$  years (mean  $\pm$  SD); range 50-74 years.

Healthy volunteers across the adult age range were recruited from the volunteer database at the Wellcome Department of Imaging Neuroscience. Twenty-seven healthy volunteers participated in the behavioural study. Twenty healthy volunteers participated in the fMRI study, of whom sixteen had already participated in the behavioural study. Details of healthy participant demographics are given separately within the behavioural and fMRI methods sections below.

**Table 1.** Patient characteristics

Patient	Sex <sup>a</sup>	Age (years)	Site of lesion <sup>b</sup>	Time since stroke (months)	Medications
1	M	63	Right MCA	42	Aspirin, citalopram, simvastatin
2	M	59	Right anterior MCA	28	Bisoprolol, flecainide acetate, simvastatin, warfarin
3	F	62	Right posterior MCA	156	Amlopodine, aspirin, atenolol
4	F	74	Left subcortical	1.5	Amlopidine, aspirin, dipyridamole, ramipril, simvastatin
5	M	50	Left subcortical	61	Aspirin, indapamide hemihydrate, perindopril, simvastatin
6	F	60	Left partial MCA	28	Levothyroxine, simvastatin, warfarin
7	F	51	Left MCA	84	Aspirin, dipyridamole, simvastatin
8	M	60	Brain stem infarction affecting right hemisphere	4.5	Amlodipine, aspirin, bendroflumethiazide, dipyridamide, lansoprazole, simvastatin

<sup>a</sup>M = male; F = female    <sup>b</sup>MCA = middle cerebral artery

With the exception of one patient (patient 2), all participants were right handed according to the Edinburgh handedness scale (Oldfield, 1971) (premorbidly for the patients with right-sided impairments). All participants reported no history of drug or alcohol abuse. The healthy volunteers reported no neurological illness, psychiatric history, vascular disease or hypertension, and were not taking regular medication. Two patients (patients 6 and 7) reported suffering with post-stroke depression.

The study was approved by the Joint Ethics Committee of the Institute of Neurology, UCL and National Hospital for Neurology and Neurosurgery, UCL Hospitals NHS Foundation Trust, London (Appendix A). Full written consent was obtained from all participants in accordance with the Declaration of Helsinki (see Appendices B to E for copies of healthy volunteer and patient information sheets and informed consent forms).

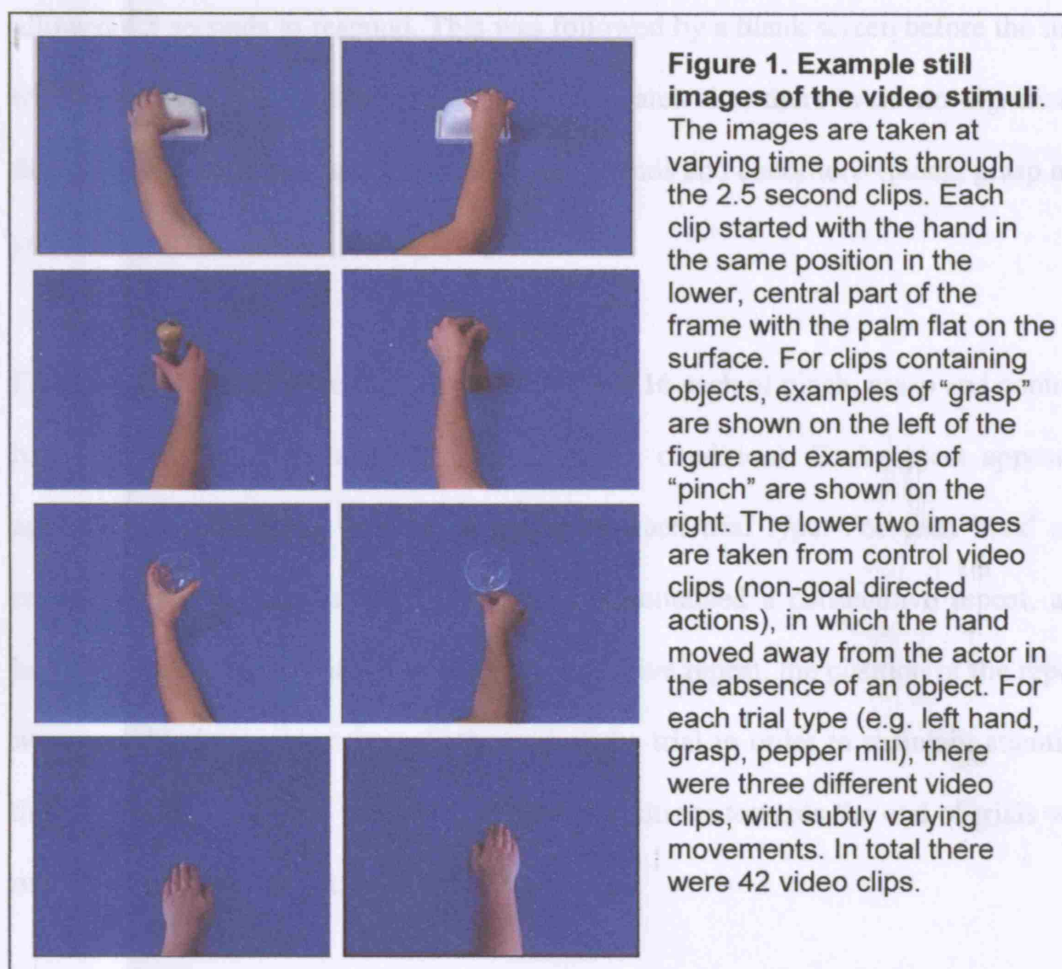
### ***2.1.2 Stimuli***

For both the scanning and behavioural studies, video stimuli were developed for use in the one-back task. Video clips were recorded using a digital camcorder, and digitally edited using Adobe Premiere version 6.0 (The Mathworks Inc., USA). They were saved in the highest resolution without compression.

The experimental video clips (“goal directed actions”) showed a hand interacting with an object (wine glass, pepper mill or butter dish). Functional, everyday objects were selected as stimuli in order to maximise the ecological validity of the study. The hand interacted with the object using either a precision grip (“pinch”), involving only the thumb and index finger, or a whole hand grip (“grasp”), involving the palm of the hand and all five fingers. This was to enable comparisons between observation of movements that were within the stroke patients’ repertoires (grasp), with more dextrous movements that were less likely to be within the patients’ repertoires (pinch). The control video clips (“non-goal directed actions”) showed a moving hand, without an object.

The video clips were filmed against a blue background, and from an egocentric perspective in order to maximize resonance with the observer. Lighting was adjusted to minimise shadow, and the lighting conditions remained constant across video clips. The model used in the video clips was a female researcher. Jewellery and nail varnish were removed and nails were kept short in order that her hands appeared as gender-neutral as possible. For all videos, the hand began in the same position, palm down on the surface. In the experimental clips, the object was positioned centrally in the frame, a short distance from the hand. The hand then moved towards the object before picking it up in a vertical direction using either a pinch or grasp, and the clip terminated a few frames after the object was lifted from the surface. In the control video clips, the hand moved straight forwards (away from the actor) with the hand remaining palm down on the surface.

For each object and trial type (grasp/pinch/control), there were three video clips of subtly varying movements on each hand. These included one slow movement, one fast movement, and one movement at a medium speed, with an exaggerated grasp in the case of the objects (e.g., fingers opening more widely before gripping, and a slight pause before lifting). Thus, in total there were 42 2.5 second video clips, of which half involved the left hand and half involved the right hand. Examples of static images from the video clips are shown in Figure 1.



### 2.1.3 One-back paradigm

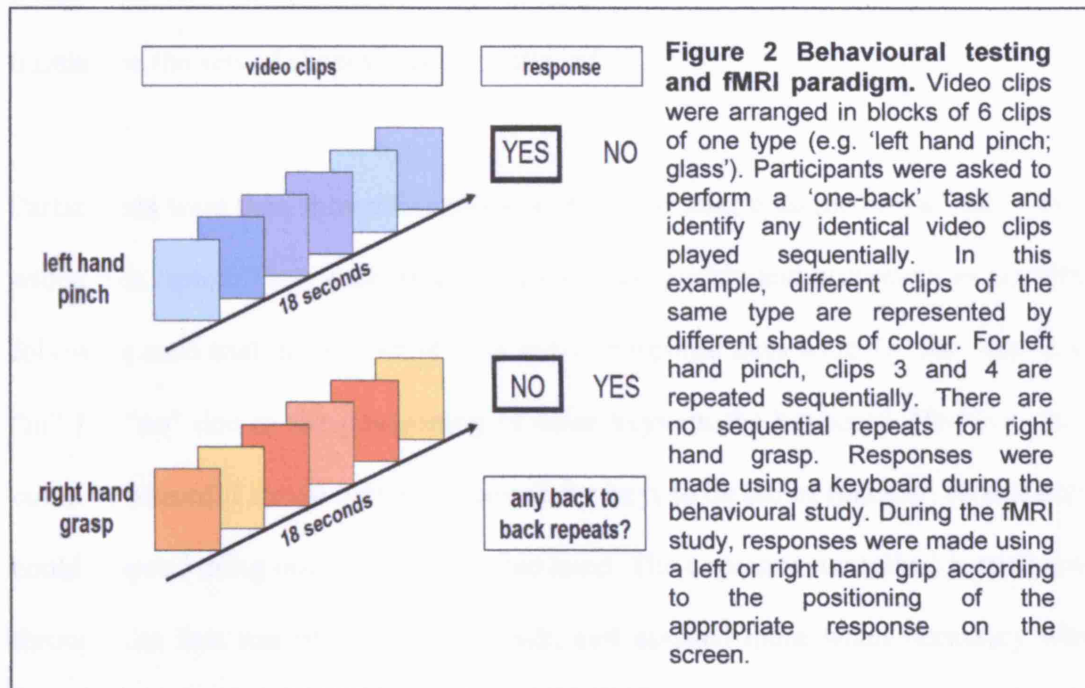
MATLAB version 6.0 (The Mathworks Inc., USA) was used to programme and run the experiment. The video clips were arranged so that one trial consisted of six video clips of the same type (e.g. same hand, grip type and object), but with subtly varying movements. Each of the three different movement types appeared twice within one trial, in varying sequences, and participants were required to detect any *consecutive* repeats (i.e., one-back task). There was a blank screen for 500 milliseconds between each clip, and each sequence of six videos was followed by a screen displaying the question "Were there any back to back repeats?" (Figure 2). Participants were

allowed 4.5 seconds to respond. This was followed by a blank screen before the next trial began. Piloting of the video stimuli indicated that there were no significant differences in accuracy rates between objects, hands and conditions (pinch, grasp and control).

For each participant, there were 48 trials in total (16 each of pinch, grasp and control; half left hand and half right hand within each condition). Each object appeared approximately the same number of times for each trial type. For each hand and condition (pinch/grasp/control), half the trials contained a consecutive repeat, and half did not. On those that did contain a consecutive repeat, the position of the repeat was more likely to occur towards the end of the trial in order to maintain attention throughout the trial. This weighting of repeat positions towards the end of trials was matched across the conditions.

The 48 trials were divided into two blocks of 24 (each lasting approximately 10 minutes) in order to minimise fatigue. Trials were arranged in a randomised order, so that the same trial types (e.g. the same object) rarely appeared twice in a row. Three playlists were created with different random sequences of trials. The playlists used and order in which the two blocks of the playlist were presented was counterbalanced across participants, in order to eliminate any confounding effects of sequence.





### 2.1.4 Training

Participants for both the behavioural and fMRI studies received the same training, although those who participated in the fMRI study received additional training as detailed in the fMRI study procedures. Participants were initially trained on a simpler version of the task in which each trial involved viewing a set of six shapes rather than six video stimuli. Participants were shown sets of six shapes printed on a sheet of paper (a total of six different sets were available). The shapes within each set of six were presented consecutively using a window cut from an overlaying sheet of cardboard. At the end of each series of six shapes, participants were verbally asked to recall if any two consecutive shapes had been identical. If they had difficulty with this, they received further explanation and training. Once it was clear that individuals

understood the concept of the one-back task and were able to respond correctly, training on the sets of shapes was discontinued.

Participants were then shown five trials of the video task, presented on a Dell 17 inch widescreen laptop. They were asked to respond as quickly and accurately as possible following each trial, using a single key press. Response keys were “z” for “yes” and “m” for “no” due to the positioning of these keys on the keyboard. However, this could be altered if stroke patients required the keys to be closer together, so that they could respond using only their unaffected hand. The experimenter talked participants through the first run of the training task, and assisted them when necessary with identifying any repeats within each trial. Following this, participants were shown the five training trials again in order to practice the task unassisted. Each participant was allowed a maximum of two runs on the unassisted training task.

### ***2.1.5 Motor functioning measures***

Motor functioning was evaluated for all participants using a variety of standard motor functioning measures:

- Nine Hole Peg Test (NHPT; Mathiowetz, Volland, Kashman, & Weber, 1992) – Timed test of fine motor coordination, involving placing 9 dowels in 9 holes using a precision (pinch) grip. The task was performed three times for each hand, and mean pegs per second scores were calculated.
- Box and Blocks (Mathiowetz, Volland, Kashman, & Weber, 1985) – Measures manual dexterity and gross motor coordination, and involves transferring small

wooden cubes from one side of a box to another across a partition, using a precision grip. The number of blocks transferred in a minute for each hand was recorded.

- **Grip Strength** – Grip strength was measured in kilograms for both hands, using a hand grip dynamometer. The maximum reading for the three attempts on each hand was recorded.
- **Action Research Arm Test (ARAT; Lyle, 1981)** – Measures gross upper limb motor functioning as well as pinch and grasp abilities. This was performed on patients only, as it can be assumed that healthy participants will score 100% on this test.

For patients, degree of impairment on each test was inferred by calculating the score on the impaired hand as a percentage of the score for the unimpaired hand.

#### ***2.1.6 Measure of spatial attention and working memory***

Cognitive impairments following stroke are common, and the one-back task requires intact cognitive functioning - particularly visual attention and working memory. If the patient group was more impaired on these factors than the healthy participants, an impaired performance on the one-back task could be due to this rather than due to a loss of motor resonance with the observed movements.

In order to assess this potential confound, the Spatial Span subtest of the Wechsler Memory Scale – 3<sup>rd</sup> Edition (WMS-III; Wechsler, 1997) was used to measure visual attention and working memory. The Spatial Span measures the examinee's ability to

hold a visual-spatial sequence of events in working memory. It consists of a forwards version, which is considered a measure of focused attention, and a backwards version, which demands more effort from working memory. Total scores on the test were converted to age-corrected, standardised scores (scaled scores) (Wechsler, 1997).

### ***2.1.7 Design***

For both studies, there were two major independent variables. The first, participant group, had three levels (stroke patients, age-matched healthy controls, and younger healthy controls). The second independent variable was observed action, which had three levels (pinch, grasp and non-goal directed control movement). Therefore, the experiment took the form of a 3x3 mixed factorial design.

For the behavioural study, the primary dependent variable was task performance, measured by percentage accuracy rates. Overall response times for each participant were also recorded as a secondary dependent variable in the behavioural study. For the fMRI study, the primary dependent variable was area and magnitude of blood oxygenation level dependent (BOLD) signal. Accuracy rates were also recorded during fMRI.

In addition, accuracy was examined in relation to scores on the Spatial Span, and both accuracy rates and brain activity were examined in relation to motor functioning scores.

## **2.2 Experiment 1 – Behavioural Study**

### ***2.2.1 Power calculation***

No previous studies have used the one-back paradigm as a behavioural measure of MNS activation. Furthermore, no previous studies have compared the immediate behavioural effects of action observation between stroke patients and healthy volunteers. It is therefore difficult to estimate an effect size on the basis of previous research. However, motor interference studies in healthy individuals have shown significant behavioural effects of observing actions that are either congruent or incongruent to the actions being performed (Brass et al., 2000; Craighero et al., 2002). In the study by Craighero et al. (2002), 12 individuals participated in each of the two conditions, and the main effect of condition (reaction times for congruent versus incongruent) produced an F-value of 4.87. Using these values in a formula derived by Thalheimer and Cook (2002), the effect size of this study was 0.94. According to Cohen (1992), this can be classified as a large effect size.

In order to have 80% power to detect a large effect size using a three group ANOVA, 21 participants per group are required at  $\alpha = 0.05$  (Cohen, 1992). Although these numbers were not achieved in the current study, the numbers of participants recruited are comparable to previous behavioural studies of action observation, which have found significant effects. This includes the study by Ertelt et al. (2007), which included two groups of eight stroke patients, and used the Wilcoxin signed ranks test to detect significant improvements in motor performance following action observation therapy.

### ***2.2.2 Participants***

All eight stroke patients (age  $59.9 \pm 7.5$  years; range 50-74 years; 4 male and 4 female), and eleven age-matched healthy volunteers (age  $57.5 \pm 11.3$ ; range 42-77 years; 6 male and 5 female) participated in the behavioural study. A third group of 16 younger healthy volunteers under the age of 40 were also recruited. One individual in this group was excluded from analysis, as he reported that he was unable to perform the one-back task due to his dyslexia. The mean age of the remaining 15 healthy volunteers in this group was 26.5 years (SD 4.5, range 21 to 35, 4 male and 11 female). Independent samples t-tests revealed no significant differences in age between the patient group and the older control group ( $t(17) = .53, p > .05$ ). The mean age of the younger control group was significantly lower than both the older control group ( $t(12) = 8.64, p < 0.01$ ) and the patient group ( $t(21) = 13.48, p < 0.01$ ).

The mean number of years in education was  $18.27 \pm 1.58$ ,  $16.82 \pm 2.71$  and  $15.13 \pm 3.60$  for the younger controls, older controls and patients respectively. The younger control group was significantly more educated than the patient group ( $t(8) = 2.35, p = 0.05$ ), but there were no significant differences in education levels between the two control groups or between the older controls and the patients.

### ***2.2.3 Procedures***

Following training, participants performed the two blocks of the task on the same laptop that they had received training on. Motor functioning measures, the Spatial Span and the Edinburgh handedness scale (Oldfield, 1971) were also completed.

## 2.3 Experiment 2 – fMRI Study

### 2.3.1 *Power calculation*

Desmond and Glover (2002) have described the complexities of computing a statistical power analysis for fMRI studies. The power calculation is affected by factors such as percent signal change, intra- and inter-subject variability, and number of time points in the fMRI experiment. Simulated power curves were tested using an fMRI experiment involving a working memory task. Based on these power curves, the authors suggested that for a threshold of  $\alpha = 0.05$ , 12 participants are sufficient to ensure 80% power at the single voxel level for typical activations.

A long history of neuroimaging has shown that the numbers recruited for this study are appropriate for this type of research question. Most action observation fMRI studies have recruited similar numbers to those recruited in this study. For example, significant effects were found in Calvo-Merino et al.'s (2005) study comparing brain activation in two groups of ten dancers. There have been no other studies directly examining the neural correlates of action observation in stroke patients, but the study by Ertelt et al. (2007), which investigated motor network activation before and after action observation therapy, recruited eight stroke patients per group and found significant within and between subject effects. In a study investigating motor network activation during action observation in individuals born without hands, two aplastic patients were compared with 16 healthy volunteers (Gazzola et al., 2007).

### ***2.3.2 Participants***

Participants were excluded from the fMRI study if they had any contraindications to MRI scanning, such as metal implants or claustrophobia. Although all eight stroke patients participated in the study, one patient (patient 8) was excluded from analysis due to excessive head movements during scanning. Therefore, seven patients were included in the analysis (age  $59.9 \pm 8.1$ ; range 50-74 years; 4 male and 3 female). Nine age-matched healthy volunteers (age  $59.8 \pm 10.9$ ; range 46-67 years; 6 male and 3 female) and eleven younger healthy volunteers under the age of 40 (age  $25.5 \pm 4.8$  years; range 21-35 years; 5 male and 6 female) also participated.

Independent samples t-tests revealed no significant differences in age between the patient group and the older control group ( $t(14) = .02, p > .05$ ). The mean age of the younger control group was significantly lower than both the older control group ( $t(10) = 8.79, p < 0.01$ ) and the patient group ( $t(16) = 11.45, p < 0.01$ ). There were no significant differences in education levels between the three groups (mean number of years in education  $17.91 \pm 1.58$ ,  $17.33 \pm 2.45$  and  $14.71 \pm 3.68$  for younger controls, older controls and patients respectively).

### ***2.3.3 Procedures***

Participants were screened for contraindications to MRI scanning, such as claustrophobia and the presence of metal in the body. MRI scanning procedures were explained and all metal objects were removed from participants before entering the scanning room.



All participants had already completed the behavioural study, with the exception of four individuals who were given additional training if required. For all individuals, further training was given before entering the scanner. Although the same paradigm was used in both studies, responses were recorded using MRI compatible dynamometers rather than the keyboard during fMRI. Participants were required to squeeze these dynamometers, which consisted of two force transducers, in order to respond to each trial. As the degree of effort involved in squeezing the dynamometers varied according to participants' impairments, this variation was controlled for by asking participants to respond at only approximately a quarter of their own maximum hand grip, as measured before entering the scanner (this paradigm has previously been described by Ward and Frackowiak, 2006).

During the one-back task for the fMRI experiment, the screen showing the question "Were there any back to back repeats?" also included the words "Yes" and "No" at the bottom of the screen. The positioning of these words varied so that sometimes the "Yes" was on the left of the screen and the "No" was on the right, and on other trials this was reversed. This positioning varied randomly. Participants were given a dynamometer to hold in each hand, and were instructed to respond by squeezing the appropriate dynamometer according to where their chosen response was positioned on the screen. For example, if they believed a particular trial contained a consecutive repeat, and the "Yes" for that trial was positioned on the right-hand-side of the screen, they would have to squeeze the dynamometer in their right hand. By randomly varying the position of the "Yes" and "No", participants had to continue to attend to the task. They were also prevented from preparing their responses before

the question appeared, which would result in the activation of brain areas involved in motor preparation, as well as areas involved in action observation.

After explaining these procedures for responding, a practice task of five trials on a computer outside of the scanning room was administered. The experimenter talked participants through the practice task, and assisted them when necessary with identifying repeats and responding appropriately using the dynamometers. Following this, participants were shown the five training trials again in order to practice without assistance. Further practice runs were allowed until the participant understood the procedures for responding.

Participants were then positioned supine in the scanner with a dynamometer in each hand. They were provided with earplugs and headphones to protect them from the scanner noise, and were given access to a panic button, which they could press to stop the experiment and be removed from the scanner. A further run of the practice task was allowed in the scanner so that participants could become accustomed to responding during scanning. Functional MRI scans were then obtained while the two blocks of the task were run, and this was followed by a structural MRI scan.

#### ***2.3.4 Data acquisition***

A 3T Siemens ALLEGRA system (Siemens, Erlangen, Germany) was used to acquire both  $T_1$ -weighted anatomical images and  $T_2^*$ -weighted MRI transverse echoplanar images (EPI) (64 x 64 3 x 3mm pixels, time between slices (TE) = 30ms) with blood oxygenation level dependent (BOLD) contrast. Each echoplanar image

comprised 48 2mm thick axial slices taken every 3mm, hence there was a 1mm gap between slices. The slices were positioned to cover the whole cerebrum, with an effective repetition time (TR) of 3.12 seconds per volume. In total, 225 volumes were acquired during each of the two blocks of the task. The first five volumes of each block were discarded to allow for  $T_1$  equilibration effects.

### ***2.3.5 Data pre-processing***

Imaging data were analysed by Dr. Nick Ward using Statistical Parametric Mapping software (SPM5; Wellcome Department of Imaging Neuroscience, 2008) implemented in Matlab version 6.0 (The Mathworks Inc., USA) (Friston, Holmes et al., 1995; Worsley and Friston, 1995). Pre-processing of the data involved four stages:

*Movement correction* - For each individual, the slices within each volume were realigned to correct for movement. Patient 8 was removed from the data analysis due to excessive head movement. No other participant moved more than 2 mm in any direction, although some of this movement was task-related. In order to remove some of the unwanted movement related variance without removing variance attributable to the motor task, realigned images were processed using the 'unwarp' toolbox in SPM5 (Andersson et al., 2001).

*Slice-time correction* - The statistical model assumes all slices within each volume were taken simultaneously. In order to correct for the time offset between slices, all volumes were slice-time corrected by adjusting to the time of the middle slice.

*Normalising* – In order to account for anatomical variation between participants, the realigned and slice-time corrected volumes from each individual were normalised. This involved morphing them to a standard EPI template based on the Montreal Neurological Institute (MNI) reference brain in Talairach space (Talairach and Tournoux, 1998) and resampling to 2 x 2 x 2 mm voxels.

*Spatial smoothing* – Spatial smoothing corrects for the influence on each voxel of surrounding voxels, in order to account for inter-subject anatomical differences and to allow valid statistical inference according to Gaussian random field theory (Friston, Ashburner, Poline, Frith, & Frackowiak, 1995). All normalised images were smoothed with an isotropic 8 mm full-width half-maximum Gaussian kernel, and the time series in each voxel were high pass filtered to remove low frequency confounds.

### ***2.3.6 Statistical analysis***

Analysis of the fMRI data compared BOLD responses within and between groups. In addition, for the healthy participants, BOLD responses during action observation were correlated with age and in-scanner task accuracy. A detailed description of procedures for the fMRI analysis is given in Appendix F.

### 3. Results

#### 3.1 Experiment 1 - Behavioural Study

All variables were examined to ensure that the basic assumptions of parametric statistics were met. No outliers were identified, and all variables satisfied the assumption of normality using the Kolmogorov Smirnov test.

##### 3.1.1 Accuracy rates

A paired samples t-test confirmed findings from the pilot study that there was no significant difference in accuracy rates between observing left and right hands ( $t(33) = 1.71, p > 0.05$ ). Furthermore, within the patient group there was no significant difference in overall accuracy rates between observing the hand corresponding to the impaired hand, and the hand corresponding to the unimpaired hand ( $t(7) = .09, p > .05$ ). Therefore, further analysis did not distinguish between observed hands.

Mean percentage accuracy rates for observing pinch, grasp and control movements are shown in Table 2, which shows reduced accuracy in the patient group compared to the control groups, particularly for the pinch condition. A 3x3 mixed ANOVA was used to examine the effects of participant group (patients versus age-matched controls versus younger controls) and observed action (pinch versus grasp versus non-goal directed hand movement) on percentage accuracy rates. As a test of sphericity was failed ( $p < 0.01$ ), Greenhouse-Geisser statistics were used. There was no significant main effect of observed action ( $F(2,49) = 1.87, p > .05$ ) and no

significant interaction between observed action and group ( $F(3,49) = .49, p > .05$ ). However, a significant main effect of group was found ( $F(1,31) = 6.72, p = 0.04$ ). Post-hoc tests (Bonferroni corrected) revealed that this was due to both the younger and older control groups performing significantly more accurately than the patients (for both comparisons,  $p < 0.05$ ), and that there was no significant difference in accuracy between the two control groups.

As there were no significant differences between the two control groups, the younger and older controls were collapsed into one group. The absence of an age effect was further shown by the non-significant correlation between age and overall accuracy within this combined control group ( $r = -.02, p > .05$ ). A 2x3 ANOVA comparing performance between the combined control group and the patients for the three conditions again revealed a significant main effect of group ( $F(1,32) = 13.09, p < .01$ ) due to the higher accuracy rates of the control group, but no significant main effect of observed action and no interaction effect.

**Table 2.** Mean percentage accuracy rates

	Observed action (Mean (SD) percentage accuracy rates)			
	Pinch	Grasp	Control	Overall
<b>Younger controls</b> (n = 15)	64.81 (14.97)	68.19 (11.93)	73.84 (11.50)	69.13 (8.84)
<b>Age-matched controls</b> (n = 11)	67.05 (15.83)	62.50 (7.91)	69.31 (14.65)	66.36 (6.77)
<b>Combined controls</b> (n = 26)	65.76 (15.07)	65.79 (10.63)	71.93 (12.85)	67.96 (8.00)
<b>Patients</b> (n = 8)	49.76 (23.00)	56.17 (18.09)	57.70 (12.58)	54.50 (12.21)

### 3.1.2 Response times

Mean overall response times per trial (in milliseconds) for the younger controls, older controls and patients were  $735.13 \pm 262.16$ ,  $1331.68 \pm 490.48$ ,  $1597.82 \pm 553.59$  respectively. A one-way ANOVA comparing the three participant groups revealed a significant effect of overall response time ( $F(2,31) = 12.76$ ,  $p < 0.01$ ). Post-hoc tests (corrected for multiple comparisons) revealed that this was due to the younger controls responding significantly faster than both the older controls and the patients (for both comparisons,  $p < .01$ ). There was no significant difference between the patients and the age-matched control group. Across all participants, there was no significant correlation between response time and overall percentage accuracy scores ( $r = -0.28$ ,  $p > 0.05$ ).

### 3.1.3 Spatial Span

Mean total scaled scores on the Spatial Span for the younger controls, older controls and patients respectively were  $12.27 \pm 2.02$ ,  $12.40 \pm 2.46$  and  $8.63 \pm 2.93$ . As a scaled score of 9-11 represents “average” functioning, these mean scaled scores represent “high average” functioning in two control groups, and “average” (bordering on “low average”) functioning in the patient group (Wechsler, 1997). However, there was variation from these means within all three groups.

A one-way ANOVA revealed that mean total scaled scores on the Spatial Span differed significantly between the three groups ( $F(2,30) = 7.26$ ,  $p < 0.01$ ). Post-hoc tests (corrected for multiple comparisons) showed that this was due to both control groups performing significantly better on the Spatial Span than the patient group, while there were no significant differences between the control groups. This effect was maintained when comparing the collapsed control group (mean  $12.32 \pm 2.16$ ) with the patients using an independent samples t-test, i.e., the control group as a whole performed significantly better than the patients on the Spatial Span ( $t(31) = 3.87$ ,  $p < 0.01$ ).

In order to address whether the differences in task performance between groups were associated with the differences in spatial attention and working memory, total scaled scores on the Spatial Span across all participants were correlated with overall accuracy rates. There was a moderate positive correlation, but this was not statistically significant ( $r = 0.34$ ,  $p = 0.06$ ). There was also no significant correlation between Spatial Span scores and overall response times ( $r = -0.15$ ,  $p = 0.42$ ).



### 3.1.4 Motor function tests

Mean scores on the motor functioning measures are summarised in Table 3. Each motor functioning test produced a score for each individual's left and right hand. In addition, a single overall mean score on each test for each of the healthy controls was calculated (i.e., left and right hand scores were combined). For the patients, percentage functioning on each test was obtained by calculating scores on the unaffected hand as a percentage of scores on the affected hand.

**Table 3.** Motor functioning scores

	9HPT <sup>a</sup>		Box and Blocks <sup>b</sup>		Grip Strength <sup>c</sup>	
	Mean	SD	Mean	SD	Mean	SD
<b>Younger controls</b>						
<b>(n = 15)</b>						
Left hand	0.79	0.10	65.27	6.39	31.27	6.57
Right hand	0.86	0.12	70.20	7.39	32.07	7.99
Overall	0.83	0.10	67.73	6.00	31.67	6.97
<b>Age-matched controls</b>						
<b>(n = 11)</b>						
Left hand	0.67	0.06	59.36	7.00	32.73	8.45
Right hand	0.72	0.06	62.00	7.73	35.73	9.02
Overall	0.69	0.06	60.68	7.08	34.23	8.58
<b>Combined controls</b>						
<b>(n = 26)</b>						
Left hand	0.74	0.10	62.77	7.16	31.88	7.30
Right hand	0.80	0.12	66.73	8.46	33.62	8.47
Overall	0.77	0.11	64.75	7.27	32.75	7.64
<b>Patients</b>						
<b>(n = 8)</b>						
Affected hand	0.11	0.10	21.75	9.08	15.00	8.30
Unaffected hand	0.59	0.13	48.63	8.50	30.75	5.23
Percentage functioning <sup>d</sup>	22.10	27.44	47.38	23.25	45.04	15.97

<sup>a</sup> 9HPT = 9 hole peg test, measured in pegs per second. <sup>b</sup> Box and Blocks measured in blocks per minute. <sup>c</sup> Grip Strength measured in kg. <sup>d</sup> Percentage functioning = affected hand/unaffected hand x 100

### *Healthy volunteers*

In order to assess the relationship between age, motor functioning and performance on the one-back task in healthy individuals, the two control groups were treated as one combined group ( $n = 26$ ). Across the combined control group, there was a strong, significant negative correlation between age and overall 9HPT scores ( $r = .60$ ,  $p < .01$ ), and a moderate, significant negative correlation between age and overall Box and Blocks scores ( $r = -.44$ ,  $p < .05$ ). There was no significant correlation between age and grip strength ( $r = .17$ ,  $p > .05$ ).

Mean overall accuracy rates on the one-back task did not correlate significantly with scores on any of the motor functioning measures within the combined control group. Left and right motor functioning scores were also examined separately in relationship to observing left and right handed actions, and no significant correlations were found.

### *Patients*

Table 3 shows that motor functioning scores were substantially lower for patients than for healthy controls. Percentage functioning scores (Table 3) also indicate that the patients were performing at a substantially reduced level on their affected hands compared to their unaffected hands, particularly for the 9HPT which requires a great deal of dexterity.

As there were age-related differences in motor functioning within the control group, independent samples t-tests compared the patients with the age-matched control group. Scores for the affected hands in the patient group were significantly lower than the mean scores in the age-matched control group for 9HPT, Box and Blocks and Grip Strength ( $t(17) = 16.57, 10.52$  and  $4.89$  respectively, all  $p < .01$ ). In addition, scores on the “unaffected” hands in the patient group were also significantly lower than overall scores in the patient group for 9HPT ( $t(17) = 2.31, p < .05$ ) and for Box and Blocks ( $t(17) = 3.37, p < .01$ ). This may be partly due to the fact that for half the patients, the unaffected hand was their non-dominant hand, making direct comparison between groups problematic. These findings were maintained when comparing patients with the whole control group.

ARAT scores were obtained for patients only, and the mean percentage functioning on this test (affected compared to unaffected hands) was  $78.07 \pm 17.77$ . All patients achieved a score of 100% on their unaffected hands for this test. The ARAT comprises four subtests, of which the “pinch” and “grasp” subtests were most relevant to our study. Scores for these subtests on the affected hand (as a percentage of unaffected hand functioning on the same subtest) were  $56.25 \pm 33.26$  and  $88.19 \pm 13.43$  respectively, i.e. patients performed more accurately on the grasp subtest than the pinch subtest. A paired samples t-test revealed that this difference was significant ( $t(7) = 3.31, p = .01$ ).

### 3.2 Experiment 2 – fMRI Study

Apart from patient 8, no other outliers were identified in the fMRI study. Therefore, all remaining participants were included in the brain imaging analysis.

#### 3.2.1 *Healthy volunteers*

##### *Behavioural results*

For all healthy individuals across both age groups, there was a large, positive correlation between overall accuracy rates on the one-back task in and out of the scanner, and this correlation was significant ( $r = .59$ ,  $p < .05$ ). For the 20 control participants there was no correlation between in-scanner accuracy and age, or in-scanner accuracy and any of the motor scores. There were large, significant negative correlations between mean 9HPT score and age ( $r = -.61$ ,  $p < .01$ ) and mean Box and Blocks score and age ( $r = -.51$ ,  $p < .05$ ). When separating the controls into the younger and older groups, there was no significant difference in scaled scores on the Spatial Span ( $t(17) = -.16$ ,  $p > .05$ ).

##### *Effects of age on brain activation*

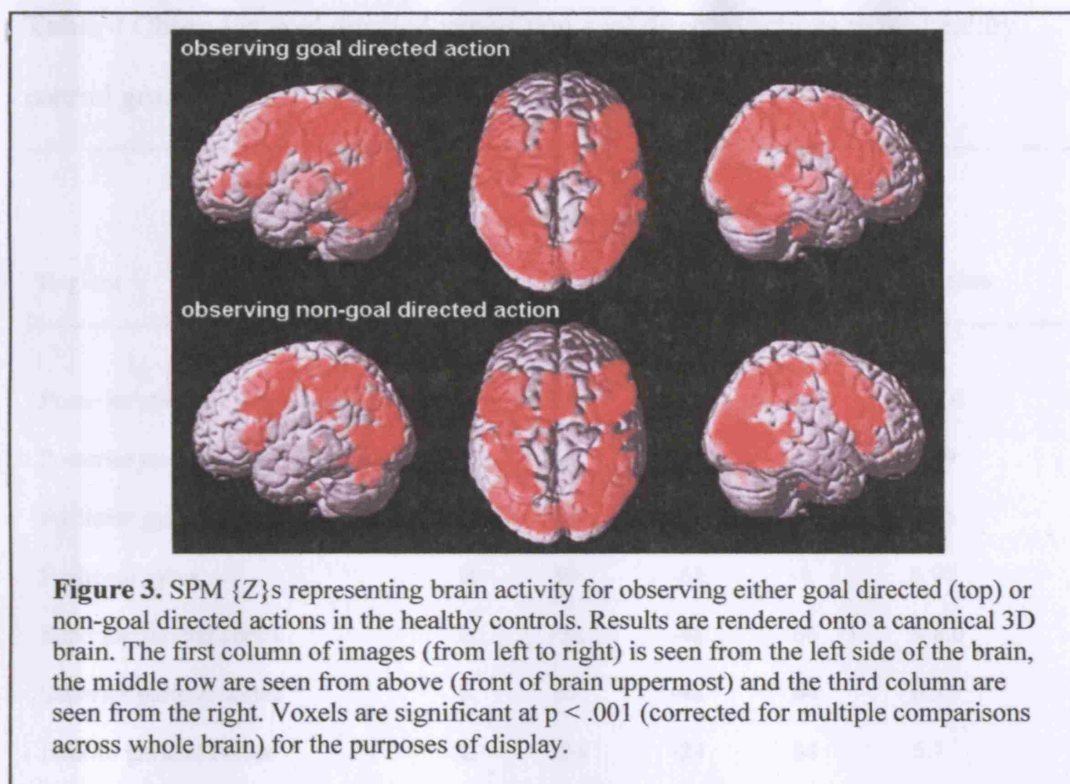
Using a two-sample t-test, no differences in brain activity could be found between the two control groups for any condition. In addition, there were no significant effects of age on brain activity for any condition using linear regression analysis

across all healthy volunteers ( $n = 20$ ). Further brain imaging analysis was therefore performed on the combined control group.

### *Effects of observing actions*

In the collapsed control group, there were no differences in brain activation between observing right and left hands, and no differences between observing pinch and grasp movements. All four of these trial types (observing left/right pinch/grasp) were therefore treated as one 'active' condition, and both control trial types (observing left/right non-goal directed actions) were treated as the control condition.

For both the active and control conditions, increased activity compared to rest was seen in a widespread bilateral network of brain regions including: dorsolateral premotor cortex (PMd) and ventrolateral premotor cortex (PMv), caudal inferior frontal gyrus (BA44), superior parietal cortex and intraparietal sulcus, supplementary motor area (SMA), anterior insula/frontal operculum, dorsolateral prefrontal cortex, primary visual cortex (area V1), lateral occipital cortex (area V5), fusiform gyrus, pulvinar, cerebellar vermis, and superior cerebellar hemispheres (lobule VI) (Figure 3).



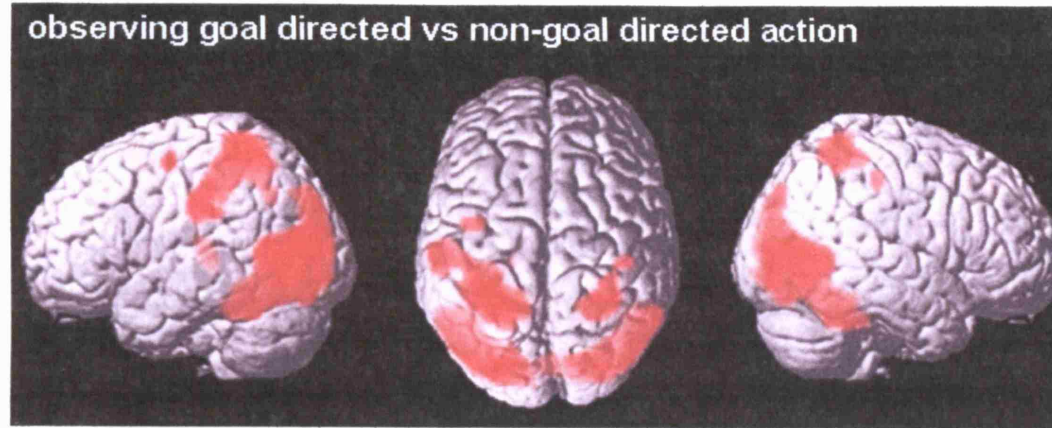
#### *Comparison between goal directed and non-goal directed actions*

When comparing observation of goal directed actions (active condition) with observation of non-goal directed actions (control condition), relative increases in brain activity were seen bilaterally in posterior middle occipital gyrus, fusiform gyrus, superior parietal cortex, both anterior and posterior parts of the intraparietal sulcus, superior cerebellum (lobule VI), as well as left inferior parietal cortex and left dorsal premotor cortex (Table 4 and Figure 4).

**Table 4** Observing goal directed versus non goal directed actions in the healthy control group

Region	Side	Talairach coordinates in MNI space			Z-value
		X	y	z	
Posterior middle occipital gyrus	L	-44	-76	6	> 8.0
Posterior middle occipital gyrus	R	46	-74	8	7.39
Fusiform gyrus	L	-28	-62	-12	6.85
Fusiform gyrus	R	30	-62	-8	5.97
Superior parietal cortex	L	-32	-48	66	> 8.0
Superior parietal cortex	R	30	-48	64	6.52
Inferior parietal cortex	L	-54	-24	34	5.7
Posterior intraparietal sulcus	L	-22	-84	30	7.37
Posterior intraparietal sulcus	R	24	-82	36	6.27
Anterior intraparietal sulcus	L	-32	-38	48	6.76
Anterior intraparietal sulcus	R	40	-28	46	5.1
Dorsal premotor cortex	L	-42	-6	56	5.24
Cerebellum (VI)	L	-26	-52	-20	6.44
Cerebellum (VI)	R	30	-52	-24	7.12

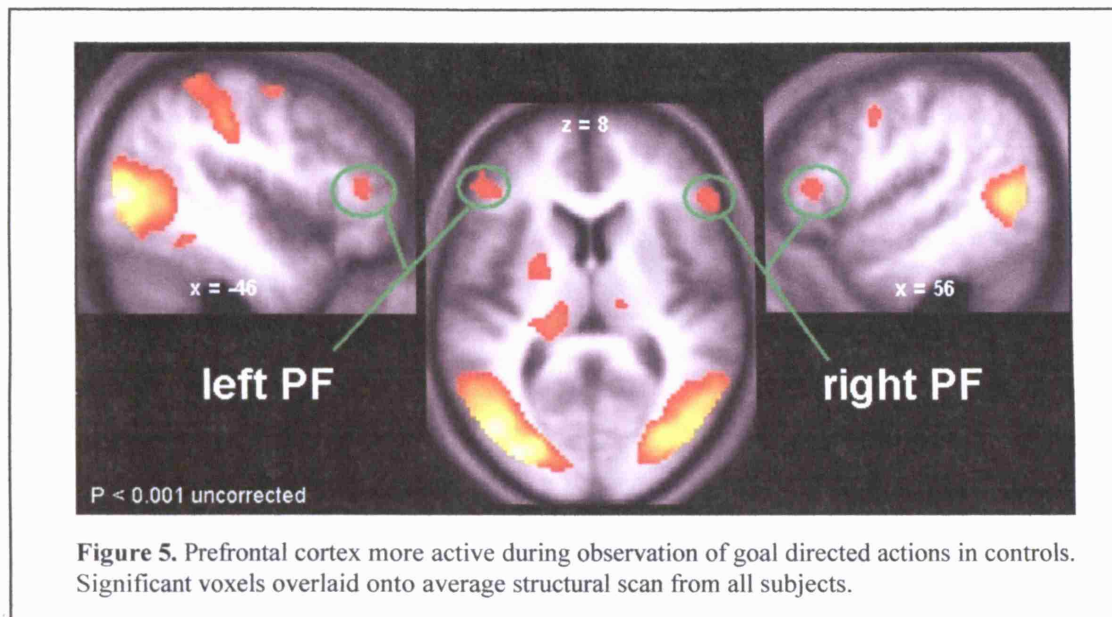
*Note.* Voxels in which there is greater activity during watching a goal directed action compared to a non-goal directed action irrespective of which hand was used to perform the action. All voxels are significant at  $p < .05$ , corrected for multiple comparisons across whole brain. Cerebellar localization performed from Schmahmann et al., 1999.



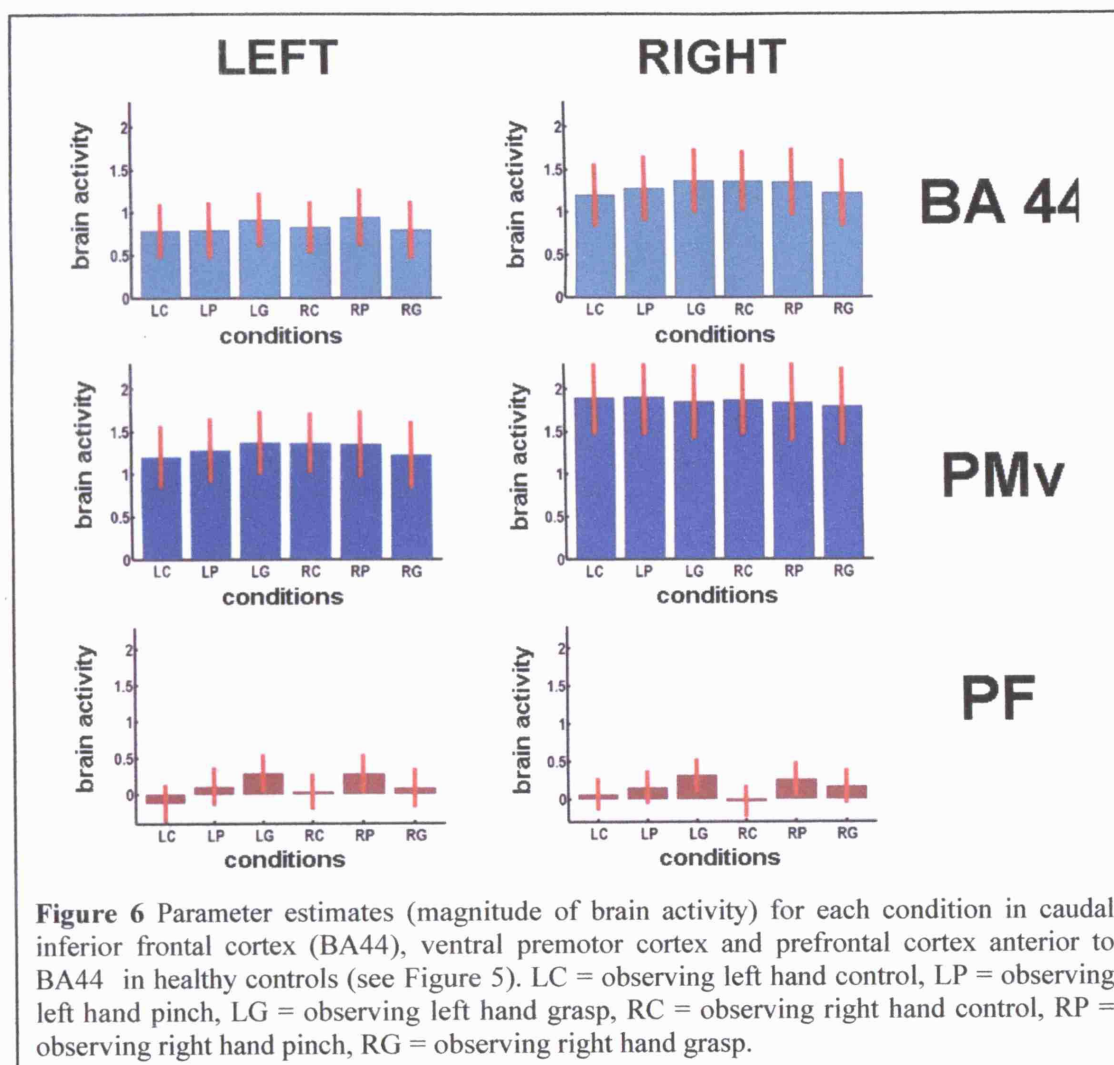
**Figure 4.** SPM  $\{Z\}$ s representing relative increases in brain activity during the observation of goal directed actions compared to non-goal directed actions in the control group. Results are rendered onto a canonical 3D brain. The first image (from left to right) is seen from the left side, the middle image is seen from above (front of brain uppermost) and the third image is seen from the right. Voxels are significant at  $p < .05$  (corrected for multiple comparisons across whole brain) as represented in Table 4.

We were interested to see whether significant differences in activity for the active compared to the control task could be seen in either ventral premotor cortex (PMv) or caudal inferior frontal gyrus (BA44) as these areas have been observed by others to be active for the observation of goal directed movements. Firstly, the threshold for significance (observing goal directed versus non-goal directed actions) was lowered to  $p < .001$ , uncorrected, revealing relative increases in activity for the observation of goal directed actions bilaterally in prefrontal cortex (PF) just anterior to BA44 (Figure 5).





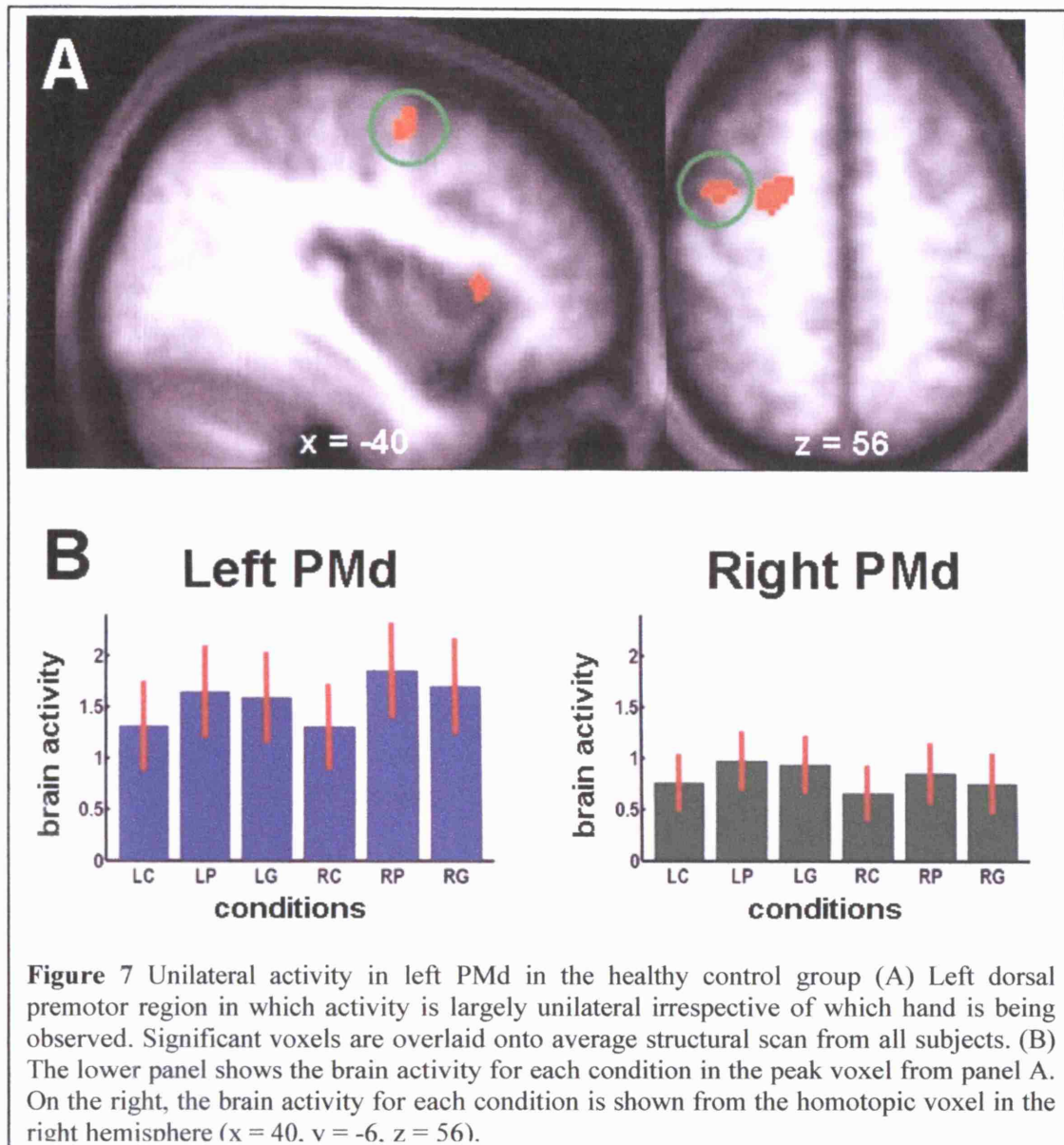
Secondly, peak voxels were defined in each region, and parameter estimates for each condition were plotted for each voxel (Figure 6). Peak voxels used were left BA44 ( $x = -56, y = 14, z = 18$ ); right BA44 ( $x = 52, y = 16, z = 12$ ); left PMv ( $x = -50, y = 4, z = 34$ ); right PMv ( $x = 48, y = 6, z = 34$ ); left PF ( $x = -46, y = 34, z = 8$ ); right PF ( $x = 56, y = 30, z = 8$ ). There were no significant differences between activity for the active and control tasks in BA44 and PMv. Although overall activity for the prefrontal regions was much lower, there was a significant difference ( $p < .001$ , uncorrected) in activity during observing goal directed compared to non-goal directed actions.



**Figure 6** Parameter estimates (magnitude of brain activity) for each condition in caudal inferior frontal cortex (BA44), ventral premotor cortex and prefrontal cortex anterior to BA44 in healthy controls (see Figure 5). LC = observing left hand control, LP = observing left hand pinch, LG = observing left hand grasp, RC = observing right hand control, RP = observing right hand pinch, RG = observing right hand grasp.

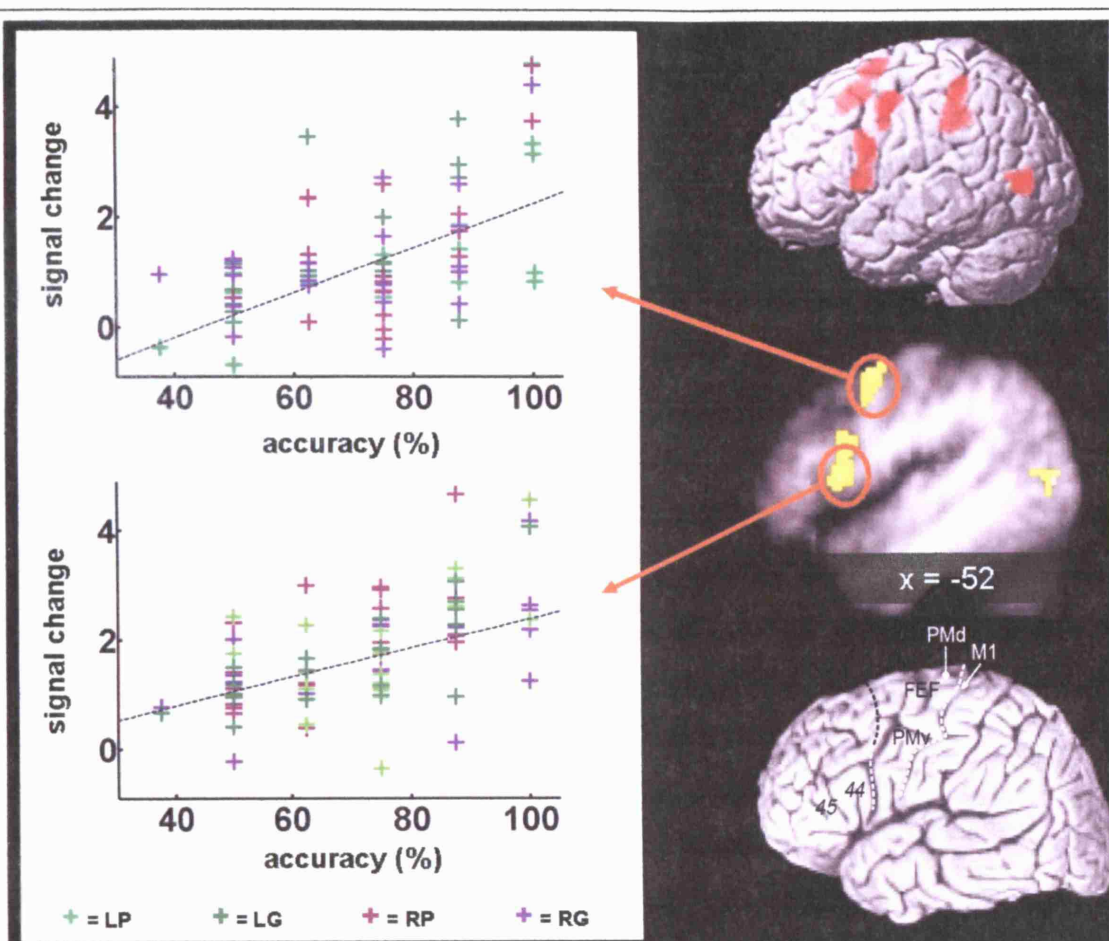
### *Laterality*

Laterality analysis (Appendix F) revealed that activity in right posterior superior temporal sulcus was unilateral ( $x = 52$ ,  $y = -54$ ,  $z = -6$ ,  $Z\text{-score} = 5.94$ ,  $p < .05$  corrected). As our primary interest was in the effects of action observation on the motor output system, a small volume correction was used to include premotor and motor cortices. Activity in dorsal premotor cortex (PMd) was significantly greater on the left side of the brain, irrespective of which hand was being observed ( $x = -40$ ,  $y = -6$ ,  $z = 56$ ,  $Z\text{-score} = 3.26$ ,  $p < .05$  corrected, cluster size = 81 voxels) (Figure 7).



### *Effects of accuracy*

Increased activity was seen in a number of brain regions with increasing accuracy, in particular left caudal inferior frontal gyrus (BA44) and dorsal premotor cortex (Figure 8), intraparietal sulcus and pre-supplementary motor area. Decreasing activity was seen with increasing accuracy in medial prefrontal and parietal cortices and left angular gyrus (Table 5).



**Figure 8** Brain regions in which activity during observation of an active task correlates with accuracy for the 1-back task for the same condition in healthy participants. Brain regions are shown rendered onto canonical brain (top right), and on sagittal slice of the average T1 weighted structural brain scan from each control subject (middle right). Voxels are significant at  $p < .0001$  uncorrected for the purpose of display. The position of motor and premotor cortical regions on the surface of the human brain is shown in the bottom right image (from Picard and Strick, 2001). Plots of signal change against accuracy are shown for left BA44 (top left) and for left dorsal premotor cortex (bottom left). Values for observing left pinch (LP), left grasp (LG), right pinch (RP) and right grasp (RG).

**Table 5** Brain activity correlating with one-back task accuracy in healthy controls

Region	Side	Talairach coordinates			Z-value	Correlation
		in MNI space				Analysis (r)
		x	y	z		
<b>(i) Positive correlation</b>						
Inferior frontal gyrus (BA 44)	L	-52	12	8	5.69	0.34
Premotor cortex (dorsal)	L	-52	-2	48	4.89	0.29
Pre-SMA	ML	-2	12	52	5.47	0.3
Intraparietal sulcus	L	-40	36	36	4.74	0.26
<b>(ii) Negative correlation</b>						
Medial prefrontal cortex	R	8	56	14	5.32	0.31
Medial parietal cortex	L	-12	-56	38	5.02	0.25
Angular gyrus	L	-48	-78	32	5.11	0.28

*Note.* Voxels in which there is a correlation between brain activity during watching a goal directed action and accuracy in the one-back task for the same condition. All voxels are significant at  $p < .05$ , corrected for multiple comparisons across whole brain. L = left, ML = midline, pre-SMA = pre-supplementary motor area, BA = Brodmann Area.

### 3.2.2 Patients

#### *Behavioural results*

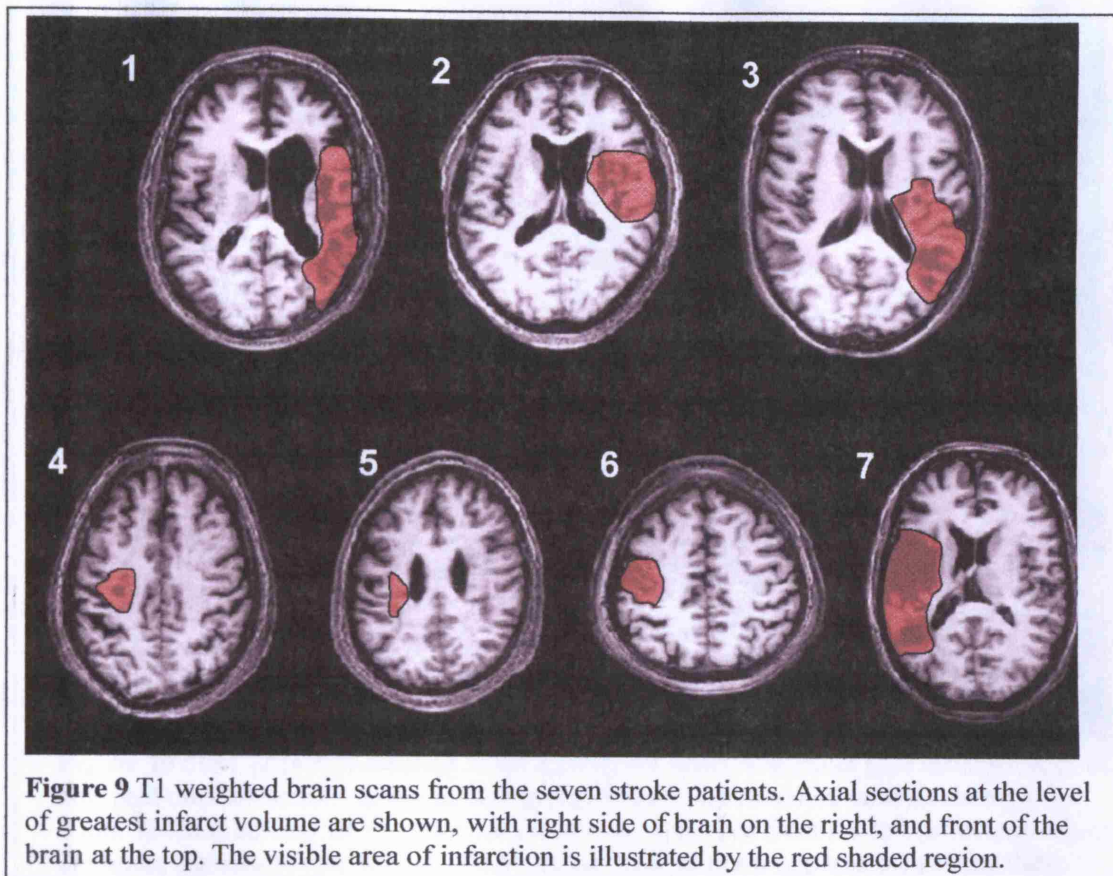
There was a large, significant positive correlation between patients' overall accuracy rates on the one-back task in and out of the scanner ( $r = .76$ ,  $p < .05$ ). Paired samples t-tests revealed that there were no significant differences in accuracy rates between observing the affected and unaffected hand for any of the conditions.



Percentage performance scores on the motor functioning tests were calculated for the seven patients (affected hand scores as a percentage of unaffected hand scores). Mean percentage performance scores were  $13.07 \pm 10.85$ ,  $42.68 \pm 15.67$ ,  $42.11 \pm 19.29$  and  $75.44 \pm 17.43$  for 9HPT, Grip Strength, Box and Blocks and ARAT respectively. On the ARAT, patients performed more accurately on the grasp subtest (mean percentage performance  $86.51 \pm 13.55$ ) than the pinch subtest (mean percentage performance  $51.59 \pm 32.98$ ). A paired samples t-test revealed that this difference was significant ( $t(6) = 3.29$ ,  $p < .05$ ). The mean total scaled score on the Spatial Span was  $8.86 \pm 3.08$ .

#### *Structural scans*

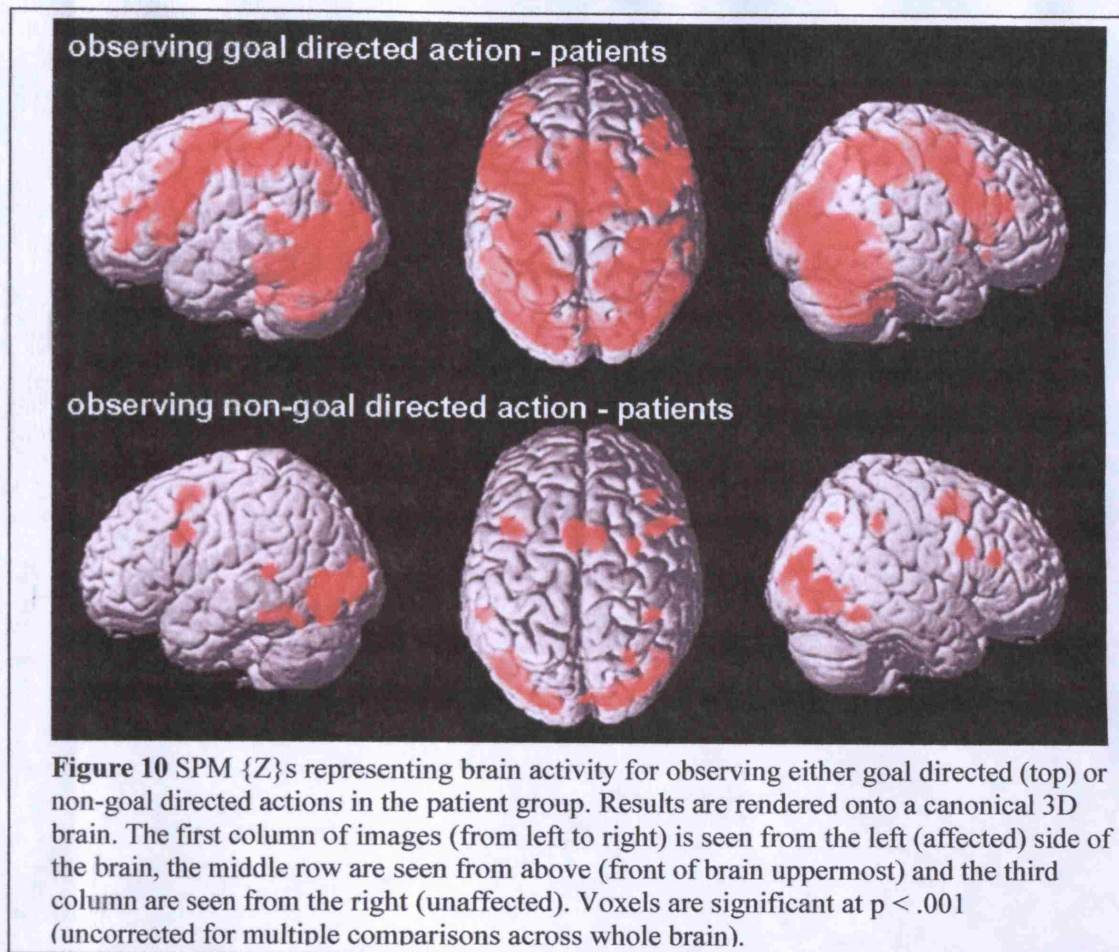
Structural scans for the seven patients included in the fMRI analysis are shown in Figure 9 (lesion sites are described in Table 1).



### *Imaging results*

The subsequent results are obtained from brain images in which the affected hemisphere is assumed to be on the left in order to average across subjects. Brain images of the 3 patients with infarcts in the right hemisphere were flipped about the mid-sagittal line.

Within the patient group, there were no overall differences in brain activation between observing affected versus unaffected hands, left versus right hands, or pinch versus grasp. The pattern of brain activity for observing active movements and for observing control movements were similar to those seen in healthy volunteers and are illustrated in Figure 10.



When comparing observation of goal directed actions (active condition) with non-goal directed actions (control condition), relative increases in brain activity were seen bilaterally in superior parietal cortex, dorsal premotor cortex and cerebellum (Table 6).

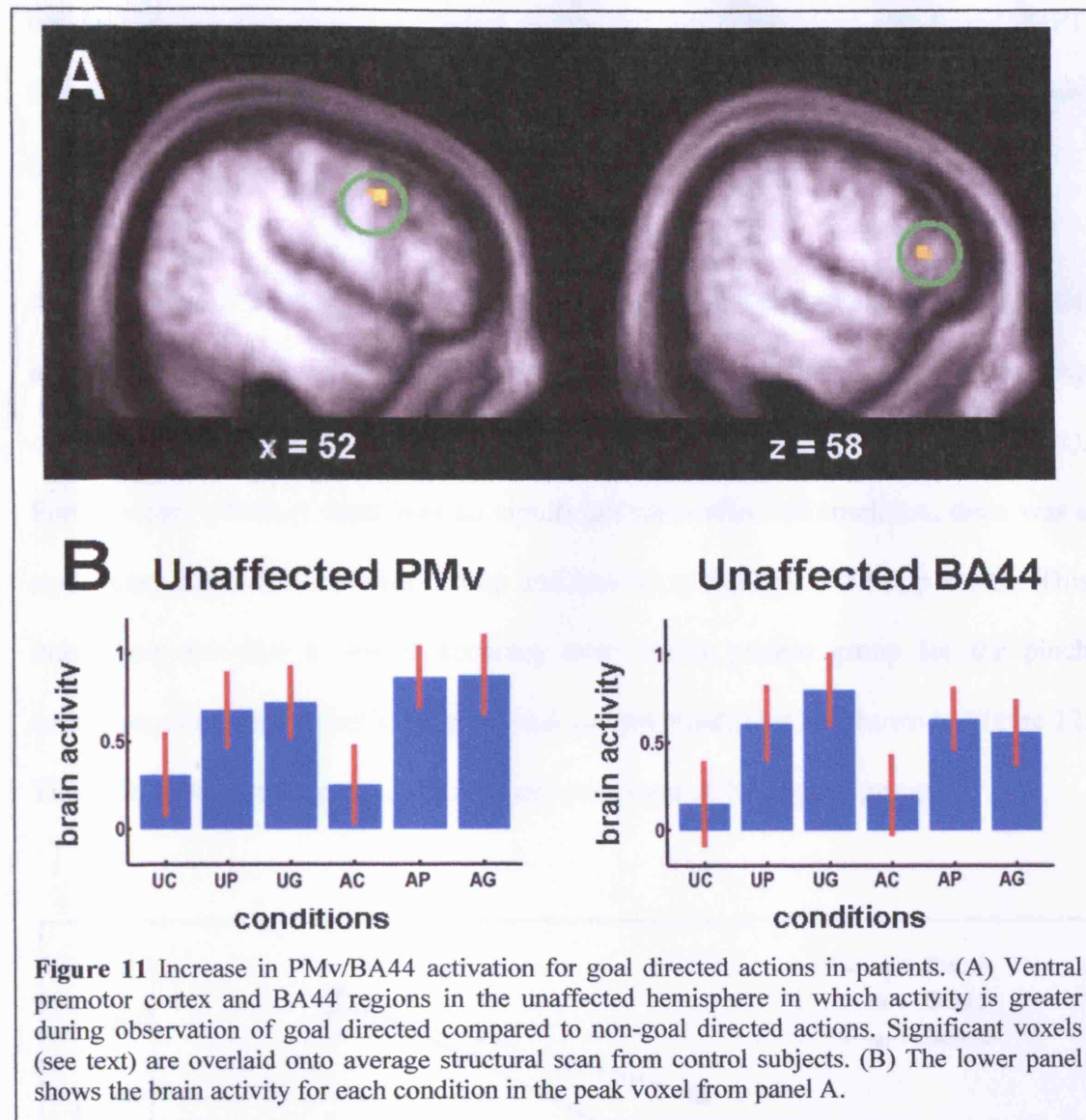


**Table 6** Observing a goal directed versus non-goal directed action – patients

Region	Side	Talairach coordinates			Z-value
		in MNI space			
		x	y	z	
Superior parietal cortex	L	-22	-48	60	4.27
Superior parietal cortex	R	36	-38	54	3.38
Dorsal premotor cortex	R	28	-8	58	3.67
Dorsal premotor cortex	L	-22	0	46	3.86
Cerebellum (VIII B)	L	-24	-50	-50	4.05
Cerebellum (VIII B)	R	14	-62	-46	3.66

*Note.* Voxels in which there is greater activity during watching a goal directed action compared to a non-goal directed action irrespective of which hand was used to perform the action for patients. All voxels are significant at  $P < 0.001$ , uncorrected for multiple comparisons across whole brain. Cerebellar localization performed from Schmahmann et al., 1999.

As with healthy volunteers, we were interested to see if any differences in activity between observing a goal-directed versus non-goal directed action were present in PMv or inferior frontal gyrus (BA44). Relative increases in activity were seen for observing goal directed actions in PMv ( $x = 52$ ,  $y = 10$ ,  $z = 38$ , Z-score = 2.73) and BA44 ( $x = 58$ ,  $y = 20$ ,  $z = 14$ , Z-score = 2.57) (see Figure 11).



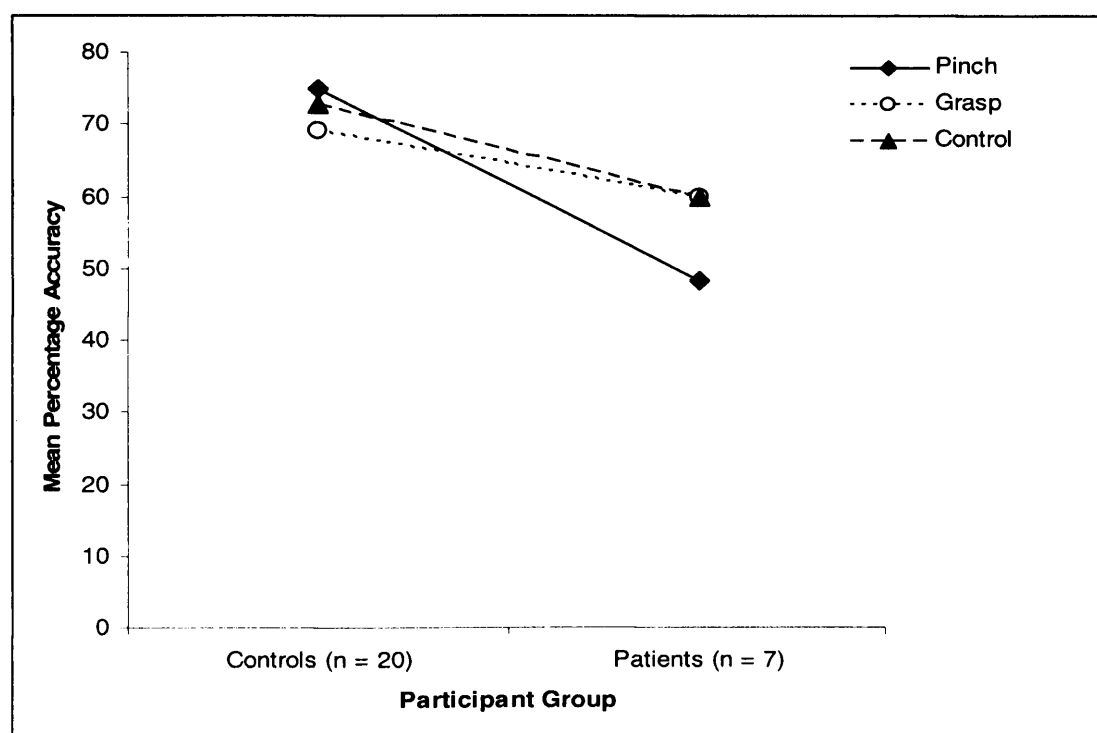
### 3.2.3 Between-groups comparisons

#### *Behavioural results*

Independent samples t-tests comparing motor scores between patients and age-matched controls showed that scores on patients' affected hands for all three tests, as well as unaffected hands on Box and Blocks, were significantly lower than mean scores in the control group (all  $p < .01$ ). These findings were maintained when

comparing with the collapsed control group, and this comparison also found 9HPT (unaffected hand) to be significantly lower than overall 9HPT scores for the controls ( $p < .01$ ).

A 2 x 3 mixed ANOVA (patients versus all controls) was used to examine in-scanner accuracy rates. There was a significant main effect of group resulting from the controls performing more accurately than the patients ( $F(1,25) = 12.00$ ,  $p < .01$ ). Furthermore, although there was no significant main effect of condition, there was a significant interaction between group and condition ( $F(2,50) = 3.61$ ,  $p < .05$ ). This interaction was due to lower accuracy rates in the patient group for the pinch condition, compared to both the grasp and control conditions, as shown in Figure 12. These differences between conditions were not seen in the control group.



**Figure 12.** Mean percentage accuracy rates during fMRI, while observing pinch, grasp and control movements.

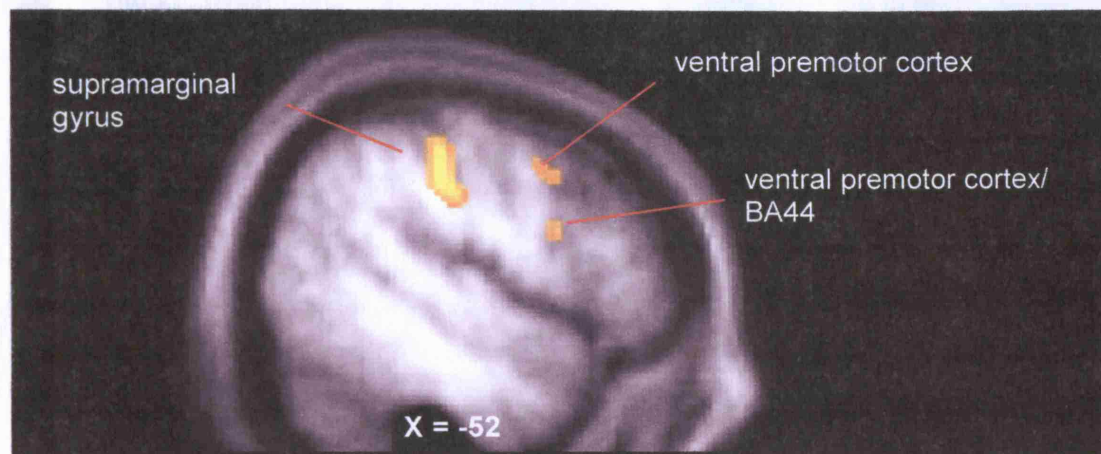
### Imaging results

Comparing average brain activity between patients and controls when observing goal directed actions revealed relative decreases in a number of brain regions shown in Table 7 (see also Figure 13).

**Table 7** Decreased activation in patients compared to controls for all active tasks

Region	Side	Talairach coordinates			Z-value
		in MNI space			
		x	y	z	
Dorsal premotor cortex	L	-36	-4	58	3.35
Ventral premotor cortex	L	-52	4	38	3.42
Inferior frontal gyrus (BA44)	L	-52	6	20	3.59
Inferior frontal gyrus (BA44)	R	48	22	28	3.35
Pre-SMA	R	8	18	46	4.39
Inferior parietal cortex	L	-52	-34	40	4.24
Middle occipital gyrus	R	34	-76	10	4.66
Middle occipital gyrus	L	-34	-86	4	3.98

*Note.* Voxels in which there is decreased activation in patients compared to controls for observing goal directed actions irrespective of which hand was used to perform the action. All voxels are significant at  $P < 0.001$ , uncorrected for multiple comparisons across whole brain.



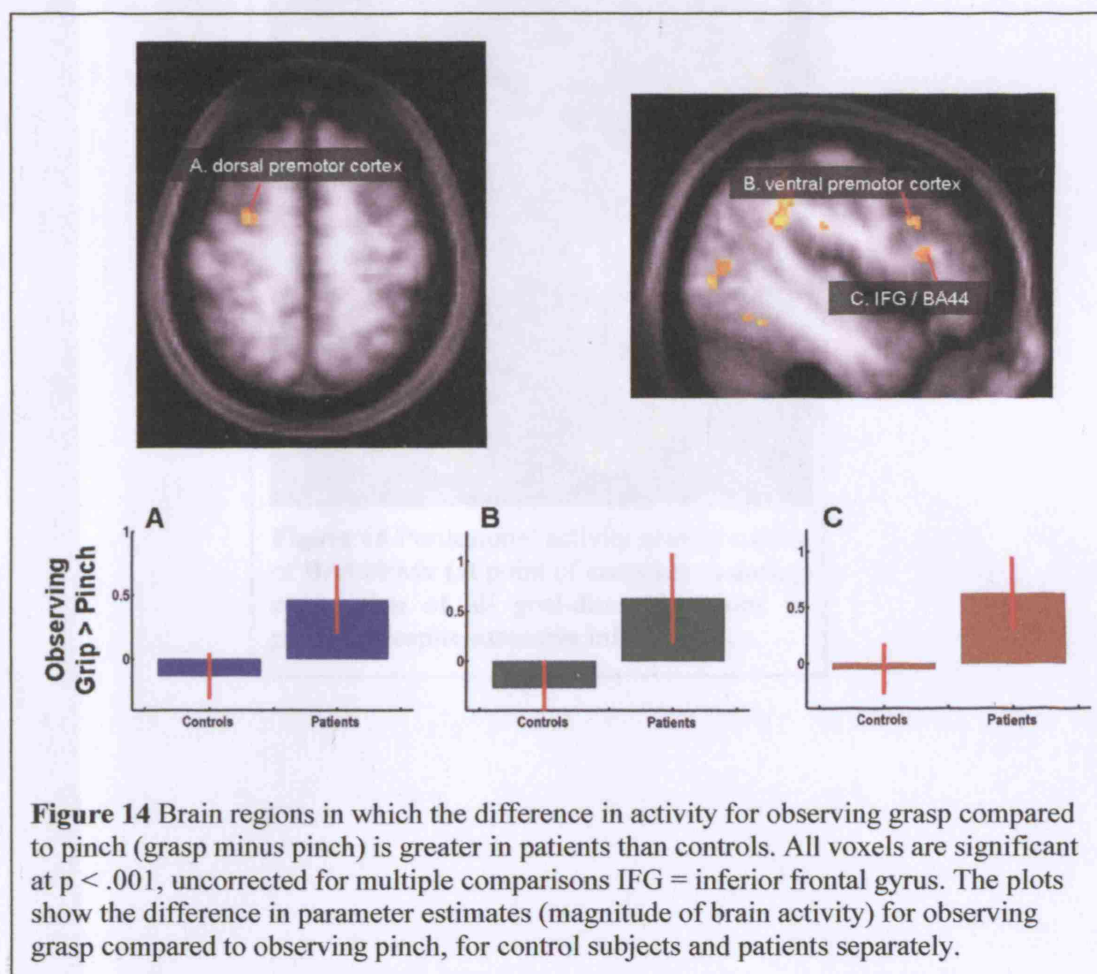
**Figure 13** Decreased activity in the affected hemisphere of patients compared to controls whilst watching goal-directed actions with either hand. All voxels are significant at  $p < .001$ , uncorrected for multiple comparisons across whole brain.

The in-scanner behavioural results demonstrated that patients were less accurate than controls at the one-back task when observing a pinch movement as opposed to a grasp. Comparing brain activation between patients and controls for these two tasks revealed significant differences in predominantly the affected hemisphere (all left hemisphere regions due to the images being flipped for analysis). Specifically, both dorsal and ventral premotor cortex, caudal inferior frontal gyrus (BA44) and supramarginal gyrus showed a greater reduction in activation for observing pinch compared to grasp in the patient group than in the control group (Table 8 and Figure 14). Thus, although on average, activity in these regions was decreased in patients for all 'active' tasks, there was a greater differential effect between observing pinch and grasp in patients than in controls.

**Table 8** Decreased activation for observing pinch compared to grasp in patients

Region	Side	Talairach coordinates in MNI space			Z-value
		x	y	z	
Dorsal premotor cortex	L	-26	2	58	3.38
Ventral premotor cortex	L	-48	14	28	3.28
Inferior frontal gyrus (BA44)	L	-48	18	16	3.13
Inferior parietal cortex (SMG)	L	-48	-42	38	3.51

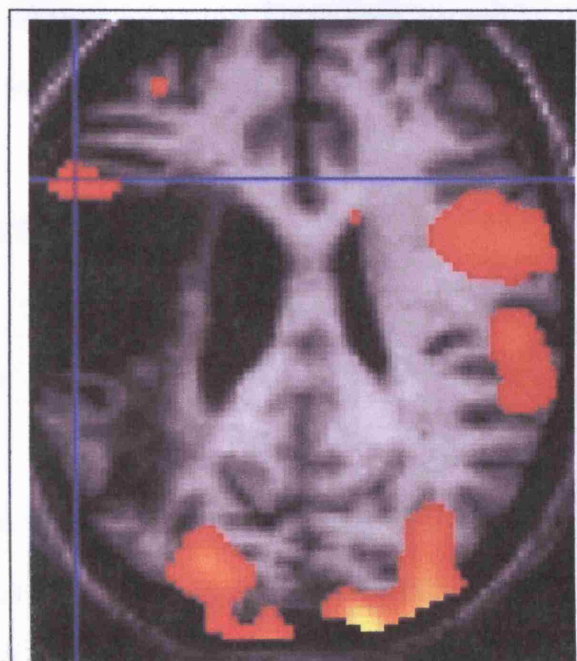
Voxels in which the difference in brain activity for observing grasp compared to pinch is greater in patients than control subjects. All voxels are significant at  $P < 0.001$ , uncorrected for multiple comparisons across whole brain. SMG = supramarginal gyrus.





*Effects of lesion location*

As we were particularly interested in BA44/PMv, patients' structural scans (Figure 9) were inspected to determine whether the overall reduction in activation in this area compared to the healthy participants could have been due to the locations of the lesions. Only one patient (patient 7) had a lesion affecting this area. Despite the extensive infarct, there was still some residual activity in BA44/PMv during this patient's observation of goal-directed actions (Figure 15).



**Figure 15** Perilesional activity seen in region of BA44/PMv (at point of cross-hairs) during observation of all goal-directed actions in patient 7 despite extensive infarct.

## 4. Discussion

This study has been the first to directly investigate the presence of a mirror neuron system (MNS) in stroke patients with motor impairments, using both a novel behavioural paradigm and fMRI. We have replicated and extended previous neuroimaging findings in healthy individuals, and we have demonstrated that action observation activates a network of “mirror” areas in stroke patients, though to a lesser extent than in healthy individuals. Furthermore, behavioural and fMRI findings in Experiment 2 suggest that MNS activation may be affected by the loss of motor repertoire in stroke patients. These findings will be discussed in further detail in the following sections.

### 4.1 Behavioural findings

#### *Motor resonance*

The behavioural study aimed to investigate whether a novel one-back paradigm could be used as an indirect measure of MNS activation. Previous neuroimaging studies (Calvo-Merino et al., 2005; Calvo-Merino et al., 2006; Cross et al., 2006) have shown that MNS activation depends on the observer’s motor repertoire (i.e., the individual’s resonance with the observed action). Therefore, it was hypothesised that MNS activity, as indicated by extent of brain activity and accuracy rates on the one-back task, would be preserved only for actions that patients were still able to perform. For the behavioural study, there were no significant differences between observing grasp, pinch or control movements for either group. As the patients had



impaired pinch functioning, we would have expected their reduced resonance with the observed “pinch” actions to be reflected in their accuracy scores for this condition. Although the patients showed a larger decrease in accuracy for pinch scores than grasp and control scores compared to the healthy participants, there was no significant interaction between group and condition. It is possible that although the patients could no longer perform the movements, they still had the motor representations of the movements and therefore could still resonate with them (to the same extent as grasp and control movements) while watching another individual performing them.

However, we did find a significant interaction between observed action and group for the in-scanner behavioural results during the fMRI study. This may be due to differences in the samples for the two studies, although most individuals participated in both studies. Alternatively, it is possible that it may reflect a specific training effect. During the behavioural study, the patients were performing at approximately 50 – 60% accuracy fairly consistently across conditions (Table 2), i.e. approximately chance level. However, during the fMRI study their scores for the grasp and control conditions improved to approximately 60% accuracy, while pinch responses did not improve (Figure 12). Healthy controls also showed improvement across conditions. Therefore, there may have been a training effect only for actions that participants were able to perform easily. As patients were significantly impaired in pinch functioning, they may not have learned to recognise these actions even after extended training on the task. If so, this would have important implications for rehabilitation on tasks that patients are unable to perform. It may be that further training would

lead to an increase in “resonance” with the action, and it will be important for future studies to investigate this further.

### *Spatial attention and working memory*

The reduced accuracy in patients compared to controls for all conditions may reflect the general motor impairments in the patient group, and therefore reduced resonance with all the observed movements. An alternative explanation could be that the patients had cognitive impairments resulting from their strokes that led to reduced performance on the one-back task. The task was challenging as the video sequences were short, and each of the six videos within the sequence were very similar to each other. An impairment of spatial attention and working memory could further increase the difficulty of this task, which required participants to attend closely to the sequence, hold the videos in working memory in order to compare each video with the previous one, and hold their response in memory until the end of the sequence.

Although we excluded patients with reported cognitive difficulties, results on the Spatial Span showed that patients were relatively impaired on these aspects of cognitive functioning compared to the controls, although this impairment was mild. For the younger control group, this may also be associated with their increased number of years in education, although there were no differences in education levels between the patients and age-matched controls. Despite the between-group differences, the correlation between Spatial Span scaled scores and task performance was not significant. This suggests that the patients’ reduced task performance was not associated with their reduced working memory and spatial attention abilities. It is

possible that with a larger sample size, a significant correlation may have been found. However, if the patients' reduced performance was due to reduced spatial attention/working memory, we would not expect to find the interaction effect seen in the accuracy rates for the fMRI study. In other words, we would expect performance to be reduced to the same extent for all conditions, but instead we found a larger decrease for the pinch condition.

This implies that the patients' reduced performance on the pinch task may have been due to impaired resonance with the observed movements, rather than impaired cognitive abilities. As they had difficulties performing the observed movements, they may not have been able to internalise them in the way that healthy individuals do during action observation. Therefore, this may have resulted in the patients having increased difficulty in detecting the subtle differences between the video clips.

### *Response times*

Overall response times were faster for the younger control group than the patients and the age-matched controls. However, the fact that there were no significant differences in response times between the patients and the age-matched controls suggests that this finding may have been due to age-related differences in processing speed and/or speed of motor responses. Furthermore, the lack of correlation between response time and accuracy rates suggests that response time was not a true indicator of task performance, as accuracy can be considered a more direct measure.

*Age effects*

There was no association between age and accuracy rates, despite the significant age-related slowing in motor performance within the collapsed control group. If the MNS is affected by motor functioning, we would expect an age-related decrease in task performance. However, the older control group were still able to perform all the motor tasks without difficulty, unlike the patients who were significantly impaired. Thus, the older control group would not have lost motor resonance with the observed actions, while the patients may have lost this resonance due to loss of the actions from their motor repertoires. Previous studies have shown that MNS activation depends on whether the observed actions are within the observer's motor repertoire, (Calvo-Merino et al., 2005; Calvo-Merino et al., 2006; Cross et al., 2006) rather than how easily or quickly the observers can perform the action.

The lack of age effects contrasts with the studies by Stefan et al. (2005), and Celnik et al. (2006). Stefan et al. (2005) demonstrated that habitual TMS-evoked thumb movements can change direction following action observation. In an action observation condition, participants were required to observe videos of thumb movements in the opposite direction to the observer's baseline thumb movements, for a 30-minute period. This resulted in a change in direction of observer's TMS-evoked thumb movements, though to a lesser extent than physical practice. The study was conducted on a sample of 12 healthy volunteers with a mean age of 34 (SD 7.6 years). The same research group later repeated the study on a sample of 11 older healthy volunteers with a mean age of 65 (SD 6.8 years) (Celnik et al., 2006). They found that in this sample, thumb movement directions only altered following a

combination of action observation and physical practice, but not following either condition alone.

Celnik et al. (2006) attributed the differences between these studies to the effects of ageing on action observation. However, there were no direct statistical comparisons between the two studies, and it is possible that the differences may have been due to variations between the samples other than age. The failure to find an age effect in the current study may have been due to differences in the task. Specifically, we were investigating participants' ability to distinguish between observed movements, rather than investigating changes in the observers' movements as a result of action observation. It may be that there were age-related differences in the motor output system during our study, that were not detected by the one-back task.

## **4.2 fMRI findings**

The fMRI study aimed to replicate previous findings in healthy individuals, as well as examining the neural correlates of action observation in stroke patients. Therefore, the findings for healthy controls and stroke patients will be discussed separately.

### ***4.2.1 Healthy volunteers***

#### *Age effects*

As in the behavioural study, no effects of age were found during action observation. Previous functional imaging studies have shown that in the healthy population, there

is an age-related decline in motor functioning, and motor task-related brain activation becomes more diffuse and bilateral with increasing age (Cabeza, 2001). This study has been the first to investigate age-related changes in brain activation during action observation, and has shown that despite a decline in motor functioning, there are no associated changes in MNS activation.

Again, it is possible that there were age-related differences in the motor output system during our task, which could not be detected using the behavioural paradigm or by fMRI. A TMS study would enable us to detect changes in corticospinal activity during action observation. This would imply sub-threshold changes in primary motor cortex (M1) excitability resulting in increased output to the muscles. Any age-related effects may have implications for the application of action observation as a tool in the rehabilitation of older stroke patients. However, the fact that we found no age-related changes in PMv/BA44 suggests that we would not find any age-related differences in M1 excitability, as M1 receives input from PMv/BA44.

### *Effects of observing actions*

As predicted, the observation of actions within the control group activated a widespread network of brain regions. These included dorsolateral and ventrolateral premotor cortex (PMd and PMv), caudal inferior frontal gyrus (BA44), superior parietal cortex and intraparietal sulcus, supplementary motor area (SMA), anterior insula/frontal operculum, dorsolateral prefrontal cortex, primary visual cortex, lateral occipital cortex, fusiform gyrus, pulvinar, and cerebellar areas. These areas, in particular PMv, BA44, parietal areas, and PMd, are considered to constitute the MNS

and have been shown to be active in previous action observation studies (Buccino et al., 2001; Grafton et al., 1996; Pomeroy et al., 2005; Rizzolatti and Craighero, 2004). Some of the activation would also have been related to the visual perception of the hands and objects, as well as the cognitive elements of the task.

### *Comparison between goal directed and non-goal directed actions*

When comparing observation of goal directed actions with observation of non-goal directed actions, there were relative increases in brain activity in a number of bilateral brain regions. These included bilateral posterior middle occipital gyrus, fusiform gyrus, superior parietal cortex, intraparietal sulcus and superior cerebellum, and left inferior parietal cortex and PMd. The increased activation in fusiform gyrus and occipital areas is likely to be due to the visual processing and recognition of the objects in the goal directed videos. The remaining areas have previously been shown to be parts of the mirror system, and it therefore seems that these mirror areas are activated to a greater extent by goal directed actions. However, there was no increase in activation in PMv/BA44 for goal-directed actions. We were particularly interested in these mirror areas due to their connections with primary motor cortex (M1).

Studies of primates have shown that area F5 is selectively activated for observing and performing goal directed actions (di Pellegrino et al., 1992; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996). Furthermore, a human study has shown increased activation in homologous inferior frontal cortex (BA44) during the observation of goal directed movements, compared to non-goal directed movements (Johnson-Frey et al., 2003). Our failure to find this effect may be due to differing paradigms – for

example, Johnson-Frey et al. (2003) showed static images of an object being grasped (goal directed condition) or being touched (non-goal directed condition). It is possible that in our control stimuli, participants imagined the hand was moving towards an off-screen object in order to grasp it. However, this is unlikely as the palm remained flat on the surface. It may be that the presence of an object is not as important for activating prefrontal mirror regions as has previously been assumed. Indeed, Buccino et al. (2001) have demonstrated that human parietal areas seem to be more important than prefrontal areas in differentiating between observed goal directed and non-goal directed actions. Other studies have found no difference in brain activation between observing goal directed and non-goal directed movements (Grèzes, Armony, Rowe, & Passingham, 2003; Oberman, McCleery, Ramachandran, & Pineda, 2007), and recent studies have shown that the human MNS seems to be active during the observation of complex, non-object related actions such as dancing (Calvo-Merino et al., 2005; Calvo-Merino et al., 2006; Cross et al., 2006). If the presence of an object is not required for maximising mirror activity in PMv/BA44, this would have implications for how action observation is implemented as a therapeutic technique.

However, we did find a relative increase for goal directed compared to non-goal directed actions in bilateral prefrontal cortex (PF), just anterior to BA44. PF is associated with executive functioning and working memory (Krawczyk, 2002). It is possible that participants found these trials more challenging than the control movements, which may have resulted in increased PF activation. If this was the case we would have expected their in-scanner task performance to reflect this, but there were no significant differences in accuracy between conditions. Therefore, it is



possible that we were in fact detecting BA44 activity, particularly as there has been some controversy regarding the exact cytoarchitectonic borders of this area (Amunts et al., 1999). Thus, it remains to be clarified whether the presence of an object *increases* activation in PMv/BA44 in healthy individuals, although our results indicate that this area is activated during action observation regardless of whether an object is present.

### *Laterality*

Left PMd was activated to a greater extent than right PMd, regardless of which hand was being observed. This area is an important part of the motor output system and MNS, and similar findings regarding its left lateralisation have been reported by others (e.g., Calvo-Merino et al., 2006). Activity in the right posterior superior temporal sulcus was also unilateral. This finding has also been observed by Aziz-Zadeh et al. (2006), who note that this area is not part of the mirror system as it is activated by action observation but not action execution.

### *Correlation with accuracy*

A particularly interesting finding was the association between task accuracy and brain activation. Activity in left caudal inferior frontal gyrus (BA44), PMd, intraparietal sulcus and pre-supplementary motor area was positively correlated with task accuracy during scanning. These areas are all mirror areas that are active both during action observation and action execution. The activation of BA44 is particularly important due its potential for facilitating motor output from M1. Thus,

it is possible that for action observation to be most successful as a rehabilitation technique, an ability to accurately distinguish between observed actions is important.

As discussed previously, differences in accuracy on the task may reflect motor resonance with the actions, or it could reflect cognitive differences. It is possible that participants who performed more accurately were using a strategy of mentally verbalising or labelling the observed actions in order to detect differences between them. This language component would produce activity in Broca's area, which is anatomically close to BA44 in the left inferior frontal gyrus, and which has sometimes been associated with the MNS. The ambiguity regarding the cytoarchitectonic borders of this area (Amunts et al., 1999) may mean that we were in fact detecting language-related differences in Broca's area. However, the fact that increasing accuracy also increased activation in other, more clearly defined mirror areas, suggests the activity in BA44 was related to the MNS rather than a language-based task strategy.

#### **4.2.2 Patients**

##### *Effects of observing actions*

Within the patient group, the patterns of brain activity for observing active and control movements were similar to those seen in the healthy volunteers. However, there were relative overall decreases in activation in PMd, PMv, BA44, pre-supplementary motor area, inferior parietal cortex, and middle occipital gyrus, predominantly in the affected hemisphere. This demonstrates that the MNS can be

activated, though to a lesser extent, in stroke patients who have damage to parts of the motor cortical network. Furthermore, there was some residual activation in PMv/BA44 even in the patient who had damage to these areas. Our finding of MNS activation in stroke patients replicates that of Ertelt et al. (2007), who also found that mirror areas were activated to a greater extent following action observation therapy.

The fact that PMv/BA44 can be activated to some extent in stroke patients during action observation is exciting, as this area has potential to modulate motor output from M1 to the hand and arm muscles. Neuroimaging studies including the current one have not found M1 activation during action observation. However, TMS studies have suggested that there is increased excitability in M1 during action observation in healthy individuals (Fadiga et al., 1995). It will be essential to investigate this further in order to discover if action observation can truly increase motor output in stroke patients. A TMS study of action observation in stroke patients would help us to determine whether there is increased cerebrospinal activity from M1 during action observation.

#### *Comparison between goal directed and non-goal directed actions*

Relative increases in brain activity were seen in bilateral superior parietal cortex, PMd and cerebellum, and PMv and BA44 of the unaffected hemisphere for goal directed compared to non-goal directed actions. Thus, compared to the control group, fewer areas showed an increase in activation for goal directed actions. However, the finding in patients of increased activation in PMv/BA44 for goal directed actions was not seen in the control group. This may suggest that the increase in prefrontal activity

seen in the healthy controls for goal directed actions may have in fact been activity in nearby BA44. Another possibility is that the cortical damage in the patient group led to recruitment of alternative areas. Either way, the finding that goal directed actions activate the MNS, particularly PMv/BA44, to a greater extent for patients, implies that the presence of objects should be an important aspect of action observation as a therapeutic intervention.

It may be important that the increase in activation in PMv/BA44 was in the unaffected hemisphere, as studies in healthy individuals have shown that left PMv/BA44 is a key part of the MNS (Buccino et al, 2001; Grafton et al, 1996; Pomeroy et al., 2005). However, it is possible that stroke patients with left hemisphere damage may compensate by recruiting alternative parts of the MNS, as seen in aphasic individuals during the observation of hand actions (Gazzola et al., 2007). Further studies will be necessary to determine this.

### *Motor resonance*

The in-scanner behavioural results demonstrated that patients were less accurate than controls at the one-back task when observing a pinch movement as opposed to a grasp. Interestingly, comparisons of brain activity between groups reflected this interaction effect. Predominantly in the affected hemisphere, dorsal and ventral premotor cortex, caudal inferior frontal gyrus (BA44) and supramarginal gyrus showed a greater reduction in activation for observing pinch compared to grasp when comparing the patients to the controls.

This finding supports the hypothesis that patients would show selectively reduced MNS activation for actions that they could no longer perform. It is possible that they had lost resonance with these actions, i.e. they were unable to form internalised motor representations of the observed actions due to damage to their cortical motor networks. The fact that there were no differences between observing affected and unaffected hands is perhaps surprising, although motor functioning measures showed that motor performance was reduced on average for both hands in the patient group. This may partly be due to the patients who had been forced to use their non-dominant hands following stroke. This significant overall reduction in motor performance compared to the control group may have resulted in overall reduction in resonance with the more impaired actions (i.e., pinch).

#### *Effects of lesion location*

It is important to note that the reduced activation in PMv/BA44 for the patient group is not due to absence of these structures, as there was only one patient with damage to these areas. It is therefore unlikely that the reduction in PMv/BA44 activity in the patient group as a whole was purely due to this patient's damage, particularly as she still showed some residual activation in these areas. Furthermore, if activity was decreased due to absence of these structures, we would have seen the same reduction in activity for all conditions, rather than the task-specific modulation of activity. The overall reduction in activity in this area in the patient group may instead be due to the effects of brain injury on the interconnections between cortical areas.

### 4.3 Summary and conclusions

This study has used behavioural and fMRI techniques to investigate a number of hypotheses. We have developed a novel behavioural method for assessing MNS activation, and accuracy on this task correlates with brain activity during action observation. This, and the behavioural interaction effect seen in the second study, suggests that this behavioural task is able to detect MNS activation. Although the behavioural task seems to be a less sensitive measure of MNS activation, it could potentially be used in future studies of action observation that could easily be carried out in hospital wards or in patients' homes. Unlike other behavioural techniques for measuring MNS activity (e.g., the motor interference studies by Brass et al., 2000 and Craighero et al., 2002), no motor response is required with the impaired hand. Therefore, it is suitable for use with severely impaired patients. The task may also have potential to be used as a means of engaging patients during action observation as a therapeutic intervention.

We have also replicated the findings of previous neuroimaging studies, by demonstrating that in healthy individuals a number of areas are active during the observation of actions, particularly goal directed actions. MNS activation does not appear to be affected by age, which implies that if action observation is to be utilised as a therapeutic intervention, it may be appropriate for patients of all ages.

Importantly, we have demonstrated that the MNS can be activated in stroke patients, although to a lesser extent than in healthy individuals. In particular, PMv/BA44 was activated in our patient group. This is an exciting finding, as these areas have the

potential to modulate motor output to hand and arm muscles via anatomical connections to M1. It will be important for future studies to establish whether there is increased corticospinal activity during action observation in stroke patients, as seen in healthy individuals. This would confirm that action observation can be of benefit to stroke patients as a therapeutic intervention.

We have additionally shown that compared to healthy individuals, MNS activation is reduced to a greater extent for actions that patients can no longer perform. This may reflect a reduced ability to form motor representations of those actions. This could have implications for action observation as a therapy - it may not be appropriate to target it at patients who are the most impaired, if the observed actions do not activate their MNS. However, it is these patients who could potentially benefit the most from action observation therapy, as they are unable to use physical practice as a rehabilitation technique. It is possible that with extended periods of action observation, the motor representations may be re-formed in such patients, and this will require further investigation. It will also be crucial to investigate the influence of factors such as time since stroke, lesion location, cognitive functioning and degree of motor impairment on MNS activity, in larger samples of patients. This will enable us to determine which patients are most likely to benefit from action observation therapy, in order to plan clinical trials.

In conclusion, this study has shown that action observation has potential as an intervention for stroke patients with motor impairments, and is a promising first step along the path to developing such an intervention.

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## **Part 3: Critical Appraisal**

In this appraisal I will discuss the development of the research question, methodological dilemmas, reflections on the research process, and strengths and limitations of the research. I will conclude by discussing future directions for research and the clinical implications of my findings.

### **1. Development of the research question**

Human motor cognition is an area that fascinates me. My background in dance training, together with my interest in neuropsychology, has led me to question whether existing knowledge of the control of action in healthy individuals can be applied to individuals with impaired movement resulting from acquired brain injury.

I first came across the notion of the mirror neuron system (MNS) when reading sports psychology literature about motor imagery as a technique for improving motor performance in athletes (Morris, Spittle, & Watt, 2005). This led me to the wealth of literature on motor imagery and action observation in healthy individuals. I then became interested in whether these techniques can be used to rehabilitate individuals with impaired movement due to brain injury. I focussed on stroke due to its high incidence. My search uncovered very few papers investigating motor imagery in stroke, and even fewer on action observation in stroke patients. However, these papers showed promise for utilising the MNS in stroke rehabilitation, and prompted me to investigate this area further.

Due to the criticisms of motor imagery studies (e.g., Holmes, 2007), I decided to use action observation for my own research. There has been one completed study showing positive effects of action observation therapy over a period of several weeks (Ertelt et al., 2007). However, there have been no studies directly investigating the immediate mechanisms underlying action observation in stroke patients. Before introducing a potential new treatment, it is important to have an understanding of how, why and in whom it works. Therefore, this formed the focus of my research.

Specifically, I aimed to investigate whether the MNS can be activated in stroke patients, and how this differs from findings in healthy individuals. The one-back paradigm was initially intended purely as a task to ensure participants were attending to the video stimuli during functional Magnetic Resonance Imaging (fMRI). However, it was decided that it should be included as a separate study, in order to determine whether it could be sensitive enough to detect differences in MNS activity between patients and controls.

## **2. Methodological dilemmas**

When developing the one-back task, a number of methodological dilemmas were encountered. These will be described in this section.

### ***2.1 Selecting the objects***

In order to maximise brain activation and increase the generalisability of the results, functional, everyday objects were used in the video clips. We decided to include



more than one object in order to further increase generalisability and also improve participants' attention to the task. However, deciding on which objects to use raised a number of methodological issues. After brainstorming a list of approximately 20 objects that can be interacted with using either a pinch or a grasp, objects were then excluded on the basis of the following criteria:

- Asymmetrical objects were excluded. This was to reduce the possibility of participants using the orientation of the object as a cue to identify differences between video clips, rather than focussing on the hand movement.
- Objects suspended from a string such as a camera case (which can be lifted using a pinch at the carry loop or using a grasp on the body of the case) were excluded, both due to their asymmetry and also due to the unpredictable movement of the object when picking it up by the string. This could be used as a cue during the one-back task rather than focussing on the hand movement.
- We intended to compare observation of pinch grips, which require greater dexterity, with observation of grasps, which patients would be more likely to be able to perform. Therefore, objects that involved particularly complex hand movements when grasping were excluded. For example, grasping a bunch of keys requires several small finger movements to manipulate the keys when picking them up. We also excluded a roll of sellotape as a potential stimulus. Although this can be lifted either using a pinch on the edge of the roll or by grasping the whole roll, stroke patients would find the whole hand grasp difficult to perform as it involves the fingers being outstretched before grasping.
- In order to enable a fair comparison between pinch and grasp, we included only objects that could be interacted with using either grip type to produce the same

outcome. For example, a pinch grip can be used to lift the lid of the teapot, and a grasp can be used on the handle to lift the whole teapot. However, as this achieves two different outcomes, this object was excluded from the list.

- Finally, objects were only included if it seemed natural to use either grip type on that object. For example, we initially thought that a tea cup would be a suitable object to include as it could be lifted using either a grasp on the body of the cup or a pinch at the handle. However, it would be unnatural to use only the thumb and index finger to pinch the handle, as other fingers are normally involved to support the weight of the cup underneath the handle. Therefore, this was excluded.

The remaining objects on the list were a wine glass (lifted using a pinch at the stem or by grasping the bowl), a butter dish (the lid can be lifted by pinching the knob or by grasping the lid on one side of the knob), and a pepper mill (lifted using a grasp in the centre or by pinching the knob on top of the mill).

## ***2.2 Selecting the hand movements***

The one-back task required that each experimental trial contained a series of video clips, which varied only on the basis of the hand movement. Initially, we decided to use natural variations in the hand approaching and lifting the objects. We therefore filmed our model's hand picking up each of the objects approximately 20-30 times for each object, hand and grip type. Using video editing software, the selected film sequences were then cut into approximately 2 second long clips. Six of these clips were then selected for each object, hand and condition. However, despite selecting

the most distinctive hand movements, all the clips within each trial type looked virtually identical. After informally piloting the task on staff within the department, we realised that if we used these stimuli, we would be likely to find a floor effect in the behavioural data. Participants would also be more likely to become frustrated by the task and lose concentration.

In order to resolve this problem, only the three most distinctive clips within each trial type were selected. Thus, each of the three clips would be shown twice within a trial of six clips. On half the trials the clips would be arranged so that there was a consecutive repeat (i.e. two identical clips next to each other in the sequence). Although this new design made the task easier, the selected video clips were still too similar, particularly for the pinch condition which was more difficult than the grasp condition.

We therefore considered ways in which the movement of the hand towards the object could be varied in a systematic way, rather than using natural variations. Possibilities included varying the speed, quality, or trajectory of the movement, or varying the positioning of the fingers on the object. The latter was excluded as it would result in participants focussing on finger positioning rather than the hand movements in order to complete the task. It was decided that varying the trajectory of the hand may seem unnatural. Therefore, we re-filmed the stimuli and systematically varied the speed and quality of the movements. For each trial type we selected one slow movement, one fast movement, and one movement at a medium speed, with an exaggerated grasp. Each of these appeared twice within a trial.

### ***2.3 Selecting the control stimuli***

As there were several factors in the experimental stimuli (an object, a hand, movement and a grasp), a control condition was needed in order to determine the specific effects of goal directed actions. By subtracting brain activity during the control condition from brain activity in the experimental condition, activity associated with the factor of interest can be determined. Options ranged from “low level” control conditions which matched the experimental stimuli on only one aspect, to “high level” controls that differed on only one aspect such as presence of a grasp. We had to be able to vary the control stimuli in some way so that they could be used for the one-back task. Possibilities included:

- A neutral, static object in varying positions.
- A neutral object, moving at varying speeds.
- A static hand in varying positions.
- A hand, moving at varying speeds.
- A static object (wine glass, pepper mill or butter dish) and a static hand, with the hand in varying positions.
- A static object (wine glass, pepper mill or butter dish) and a moving hand that approached the object but did not grasp it.

We decided that only one control condition should be used, in order to minimise the length of the experiment and therefore help to maintain participants’ attention. We excluded controls that involved objects, as the observation of objects without movement could potentially activate mirror areas through imagined grasping of the

objects. We also decided that the control should involve movement that matched the hand movement in the experimental conditions. This would minimise activation differences between conditions caused by eye movements. Therefore, we chose the moving hand as our control.

## ***2.4 Piloting the stimuli***

The stimuli were piloted and re-filmed a number of times on right-handed undergraduate students. This was to ensure that there was a similar accuracy rate for each of the objects, so they could be collapsed into one group during the final analysis of the data. We also hoped to achieve similar accuracy rates across conditions (grasp/pinch/control) and for left and right hands. Furthermore, we hoped to achieve an accuracy rate for healthy controls that was neither too low (chance level, i.e. 50% accuracy), or too high (ceiling effect, i.e. 100% accuracy). A summary of the pilot study findings follows:

*Pilot study 1 (n = 5):* Piloting of the original video clips (using the three most distinctive clips for each trial type) revealed an overall mean accuracy rate of 69.27% (SD 8.40). There were no significant differences in accuracy between left and right hands, or between objects. However, the grasp condition was significantly easier than the pinch condition.

*Pilot Study 2 (n = 8):* After filming the new set of video clips with systematic variations in movements, the mean overall accuracy rate was 80% (SD 9.71), and there were no significant differences in accuracy between conditions or objects.

However, observing the left hand produced significantly higher accuracy rates than the right hand. This was due to some of the left-handed actions looked awkward and therefore more easily distinguishable, as the model used was right handed. An attempt to resolve this by digitally flipping the left hand images resulted in a ceiling effect (100%) when tested on a further two participants.

*Pilot Study 3 (n = 5):* Some of the video clips from Pilot Study 2 were replaced or rearranged, to increase the accuracy on the right hand video clips, and decrease the accuracy on the left hand. The final set of video clips resulted in a mean accuracy rate of 71.23% (SD = 16.25), with no significance differences between conditions, hands or objects. Therefore, these videos were used in the final task.

### **3. Reflections on the research process**

The project was ambitious given the time constraints. In particular, it took much longer than anticipated to develop, pilot and finalise the one-back task. However, these stages of the research process are important, particularly in fMRI studies, in order to ensure that the conditions are carefully matched and neither too easy nor too challenging. Large differences in accuracy rates between hands, objects and conditions within healthy individuals would make it difficult to interpret the findings when comparing to stroke patients.

I had originally intended to only conduct the fMRI study. However, we decided that it would be interesting to conduct the one-back task as a behavioural study in its own

right. As there were also long delays in booking scanning time, I focussed on recruiting for the behavioural study. There were no difficulties in recruiting healthy individuals, and 27 controls across the age range were recruited in total.

Recruiting patients at this stage was more problematic. Most of the patients who participated were chronic stroke patients who were being seen in outpatient clinics. They were booked in at a later stage in the research process (due to the delay in booking scanning time) and participated in both studies on one day. I attempted to recruit additional patients from the wards at the National Hospital for Neurology and Neurosurgery, Queen Square. However, most of the stroke patients on the wards had major cognitive difficulties, and therefore would not have been suitable for the task. After attending ward rounds and approaching ward staff, I eventually managed to recruit two patients from the wards, who participated in the behavioural study and then the fMRI study at a later stage. One of these patients was excluded from the fMRI study due to excessive head movements.

Therefore, it was not possible to recruit as many patients as anticipated. However, although higher number of patients would have been ideal, the numbers we recruited were comparable with other similar studies. Difficulties in patient recruitment are a common problem in research, and Blanton et al. (2006) have described some of the obstacles they encountered in recruiting stroke patients for a randomised clinical trial of constraint-induced movement therapy.

## **4. Strengths and limitations of the research**

### ***4.1 Strengths***

The study has been the first to examine the immediate behavioural and neural correlates of action observation in stroke patients. The findings have shown that there is potential for action observation as a therapeutic intervention, as stroke patients show residual MNS activity despite damage to some mirror areas.

A limitation of several previous studies of action observation is that participants have been asked to passively observe movements. Therefore, there is no measure of whether they have been attending to the stimuli. The one-back paradigm in our study ensured that participants were attending to the stimuli as well as providing a behavioural measure of MNS activation.

Another advantage of the one-back paradigm is that it could potentially be used in future as a therapeutic intervention. It could be used on the wards or in an individual's home as a daily task, with a limited need for staff input. However, carefully controlled trials would be needed to establish the utility of this and the effects on the motor output system.

The study was carefully designed and controlled, and aspects such as generalisability to everyday situations have been considered by using familiar, functional objects. This will be important if this paradigm is to be used in future as a therapeutic intervention. Furthermore, the differentiation between pinch and grasp when



developing the stimuli enabled us to show that losing the ability to perform an action reduces MNS activation in patients.

The inclusion of the Spatial Span was important. The study by Ertelt et al. (2007) did not include a measure of cognitive abilities in their stroke patients, which may have affected performance on their action observation task. Cognitive impairment is common following stroke, and any future studies of MNS activation in stroke patients should take care to consider this as a potential confound when comparing with healthy controls.

#### ***4.2 Limitations***

Although the Spatial Span was included, it may also be important to consider other cognitive impairments that could affect patients' ability to attend and respond to the video clips. For example, an impairment in processing speed could impact on task performance. In addition, the task required sustained attention. Although the task was divided into two blocks to minimise fatigue and help to maintain attention, several of the participants (patients and controls) reported that they found it difficult to attend to the task for ten minutes at a time. Two of the patients reported suffering with post-stroke depression and fatigue, which are common consequences of stroke. These factors may have impacted on their ability to attend to the task for a sustained period.

Furthermore, we found a moderate correlation between Spatial Span and task performance in our 34 participants, although this was not significant ( $r = .34$ ,  $p =$

.06). In order to detect a significant correlation at a medium effect size 85 participants are required (Cohen, 1992). Therefore, we may well have found a significant effect with a larger number of participants, since the  $p$  statistic was approaching significance at the .05 level. However, as discussed in the empirical paper, if the differences between groups were entirely due to differences in cognitive abilities, we would not expect to see a differential effect in MNS activity for pinch and grasp when comparing between groups.

As described previously, the study would have benefited from a larger sample size, although our sample size is comparable to previous studies. In addition, the patient group, although relatively homogeneous in terms of impairment, contained within group differences such as length of time since stroke and lesion location. However, stroke is highly variable and it is therefore difficult to control for this. If we had a larger sample size, we would have examined correlations within the patient group of MNS activity with factors such as time since stroke, accuracy and motor scores. Due to the difficulties recruiting patients, we also included one left-handed patient. However, it can be assumed that the damage in this patient outweighed any effects of laterality due to hand dominance.

## **5. Future directions and clinical implications**

Our study showed that in stroke patients, there is activity in ventral premotor cortex and BA44 in the left inferior frontal gyrus during the observation of another's actions. This is an exciting finding due to the potential for these regions to modulate

motor output from primary motor cortex. The use of transcranial magnetic stimulation in future studies will enable us to establish whether there is an increase in corticospinal activity during action observation in stroke patients. This finding would confirm that action observation can increase motor output to muscles and therefore be of benefit to stroke patients as a therapeutic intervention.

Future studies of larger samples of stroke patients will also be important to replicate our findings. Knowledge of the mechanisms of MNS activation in stroke patients with a variety of lesions, and at varying stages of recovery, will indicate which patients are most likely to benefit from action observation therapy. Following this, clinical trials can be planned in order to determine whether action observation produces lasting change in motor functioning. It will also be necessary to establish the most effective ways of delivering action observation as therapy – for example, how long each therapy session should last and how regularly it should be administered.

The potential for action observation as a therapeutic technique opens up exciting new possibilities for applying psychological theories to the rehabilitation of physical impairments. In neurorehabilitation settings, clinical psychologists have traditionally been responsible for the cognitive and emotional aspects of brain injury, while physiotherapists have been responsible for physical rehabilitation. In order for action observation to become successful as a therapeutic intervention, it would require the combined expertise of psychologists and physiotherapists. This would enable the tailoring of such a therapy to each individual patient's physical, emotional and cognitive needs and abilities.

Finally, this consideration of individual patient needs leads me to conclude with a reminder about the broader psychological impact of stroke. This thesis has demonstrated that mental processes such as action observation and motor imagery can influence the body. Similarly, psychological factors such as mood, emotion, cognition and motivation are likely to influence patients' ability to apply rehabilitation techniques designed to improve motor functioning. Post-stroke depression is common, and losing the ability to move a limb can have enormous impact on an individual's mood and sense of identity. Stroke can also have a significant effect on relationships. It is therefore essential that these factors are taken into consideration when researching and applying new interventions. As journalist Robert McCrum has written about his own stroke,

“A stroke is half medical and physical, and half psychological and emotional.....This is an affliction that has an emotional cost. To tackle it requires emotional willpower.”

(McCrum, 2004)

## 6. References

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- Cohen, J. (1992). A power primer. *Psychological Bulletin*, 112, 155-159.
- Ertelt, D., Small, S., Solodkin, A., Dettmers, C., McNamara, A., Binkofski, F. et al. (2007). Action observation has a positive impact on rehabilitation of motor deficits after stroke. *Neuroimage.*, 36 Suppl 2, T164-T173.
- Holmes, P.S. (2007). Theoretical and practical problems for imagery in stroke rehabilitation: An observation solution. *Rehabilitation Psychology*, 52 (1), 1-10.
- McCrum, R. (2004). The impact of the disease: A personal view. In N. Losseff (Ed.), *Neurological Rehabilitation of Stroke* (pp 63–66). London: Taylor & Francis.
- Morris, T., Spittle, M., & Watt, A.P. (2005). *Imagery In Sport*. Leeds: Human Kinetics.

## **Appendix A**

**Copy of ethical approval letter**



**National Research Ethics Service**  
**The National Hospital for Neurology and Neurosurgery**  
**& Institute of Neurology Joint REC**

Dr N S Ward  
 Wellcome Advanced Fellow and Honorary Consultant  
 Neurologist  
 Institute of Neurology  
 12 Queen Square  
 London  
 WC1N 3BG  
 Our Ref: 07L 212

Research & Development  
 1st Floor, Maple House  
 Ground Floor, Rosenheim Wing  
 25 Grafton Way

23 July 2007

Dear Dr Ward

**Full title of study:** Observing actions after stroke: a brain imaging and stimulation study  
**REC reference number:** 07/H0716/46

The Research Ethics Committee reviewed the above application at the meeting held on 19 July 2007

**Ethical opinion**

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation.

**Conditions of approval:-**

Regarding the Patient Information Sheet (PIS)

- The committee noted if a structural abnormality be found, recommends that the person is advised by a doctor on site, rather than informing the GP only.
- Under the heading "what is involved in the study?" mentions there are two sessions although the application mentions three sessions.

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

**Approved documents**

The documents reviewed and approved at the meeting were:

Document	Version	Date
Application	5.3	25 June 2007
Investigator C.V.	1	25 June 2007
Protocol	1	25 June 2007
Covering Letter	1	25 June 2007
Letter from Sponsor	1	28 July 2003
GP/Consultant Information Sheets	1	25 June 2007

16/46

Page 2

Participant Information Sheet PIS- Patients	1	25 June 2007
Participant Information Sheet PIS - Volunteers	1	25 June 2007
Participant Consent Form Patients	1	25 June 2007
Participant Consent Form Volunteers	1	25 June 2007
	1	25 June 2007
	1	25 June 2007
GP letter to Volunteers GP's	1	25 June 2007

**R&D approval**

The study should not commence at any NHS site until the local Principal Investigator has obtained final approval from the R&D office for the relevant NHS care organisation.

**Membership of the Committee**

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

**07/H0716/46****Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely



Chair

Enclosures:

*List of names and professions of members who were present at the meeting.*

Copy to

R&D office for UCLH



## **Appendix B**

### **Information sheet for healthy volunteers**

NB. It was verbally explained to participants that they were being asked only to participate in the behavioural and/or fMRI parts of the study. The TMS study described on the information sheet was due to take place at a later date and participants were not being asked to participate in this.







## **Appendix C**

### **Information sheet for patients**

NB. It was verbally explained to participants that they were being asked only to participate in the behavioural and/or fMRI parts of the study. The TMS study described on the information sheet was due to take place at a later date and participants were not being asked to participate in this.









## **Appendix D**

### **Informed consent form for healthy volunteers**

## WELLCOME TRUST CENTRE FOR NEUROIMAGING LEOPOLD MÜLLER FUNCTIONAL IMAGING LABORATORY

### CONSENT FORM – CONFIDENTIAL (Normal Volunteer) Version 2, August 2007

Title of project:

***Observing actions after stroke: a brain imaging and stimulation study***

**Name of Researcher:** Dr Nick Ward, Wellcome Trust Centre for Neuroimaging

UCLH Project ID number:

Centre Number:

Patient Identification Number for this study:

**Please initial box**

1. I confirm that I have read and understood the information sheet dated August 2007, version 2 for the above study and have had the opportunity to ask questions.

☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐

3. I agree to take part in the above study

☐

4. I give permission for my General Practitioner to be informed about my participation in this study.

☐

\_\_\_\_\_  
Name of Patient

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Name of Person taking consent  
(if different from researcher)

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Researcher

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

#### Comments or concerns during the study

If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Complaints Manager, UCL hospitals. Please quote the UCLH project number at the top of this consent form.

**1 form for Volunteer, 1 to be kept as part of the study documentation**



The National Hospital for Neurology and Neurosurgery is part of UCL Hospitals NHS Trust which also includes the Eastman Dental Hospital, Elizabeth Garrett Anderson and Obstetric Hospital, The Heart Hospital, Hospital for Tropical Diseases, The Middlesex Hospital and University College Hospital.



## **Appendix E**

### **Informed consent form for patients**

## Appendix F: Procedures for Statistical Analysis of fMRI Data

Statistical analysis was performed in three stages. In the first stage, data from single subjects were analysed separately using a single subject two-session fixed effects model. Each type of video was defined as a single covariate and modelled using a boxcar basis function. All hand grips (used to respond to the post-video question) were defined as a single event type and modelled as delta functions. All covariates were convolved with a canonical synthetic haemodynamic response function (HRF) and used in a general linear model (Friston, Holmes et al., 1995) together with a covariate representing the mean (constant) term over scans for each session (Figure 16).

The canonical HRF represents a typical BOLD response derived from a principal component analysis of data reported by Friston et al. (1998). Thus for each subject, voxel-wise parameter estimates for each covariate resulting from the least mean squares fit of the model to the data were calculated. The parameter estimates (or *betas*) for each covariate reflect the magnitude of increase in the BOLD signal during each condition. The statistical parametric maps of the t statistic (SPM{t}) resulting from linear contrasts of each covariate (Friston, Holmes et al., 1995) were generated and stored as separate images for each subject.

The data for the second stage of analysis comprised the pooled parameter estimates for each covariate across all subjects. Contrast images for each subject were entered into a one sample t-test for each covariate of interest. The SPM{t}s were thresholded at  $p < .05$ , corrected for multiple comparisons across whole brain.

## WELLCOME TRUST CENTRE FOR NEUROIMAGING

LEOPOLD MÜLLER FUNCTIONAL IMAGING LABORATORY

### CONSENT FORM – CONFIDENTIAL (Patient) – Version 2, August 2007

Title of project:

***Observing actions after stroke: a brain imaging and stimulation study***

Name of Researcher: Dr Nick Ward, Wellcome Trust Centre for Neuroimaging

UCLH Project ID number:

Centre Number:

Patient Identification Number for this study:

Please initial box

1. I confirm that I have read and understood the information sheet dated August 2007, version 2 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from UCLH or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree to take part in the above study. ☐
5. I give permission for my General Practitioner to be informed about my participation in this study. ☐

\_\_\_\_\_  
Name of Patient

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Name of Person taking consent  
(if different from researcher)

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Researcher

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

#### Comments or concerns during the study

If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Complaints Manager, UCL hospitals. Please quote the UCLH project number at the top of this consent form.

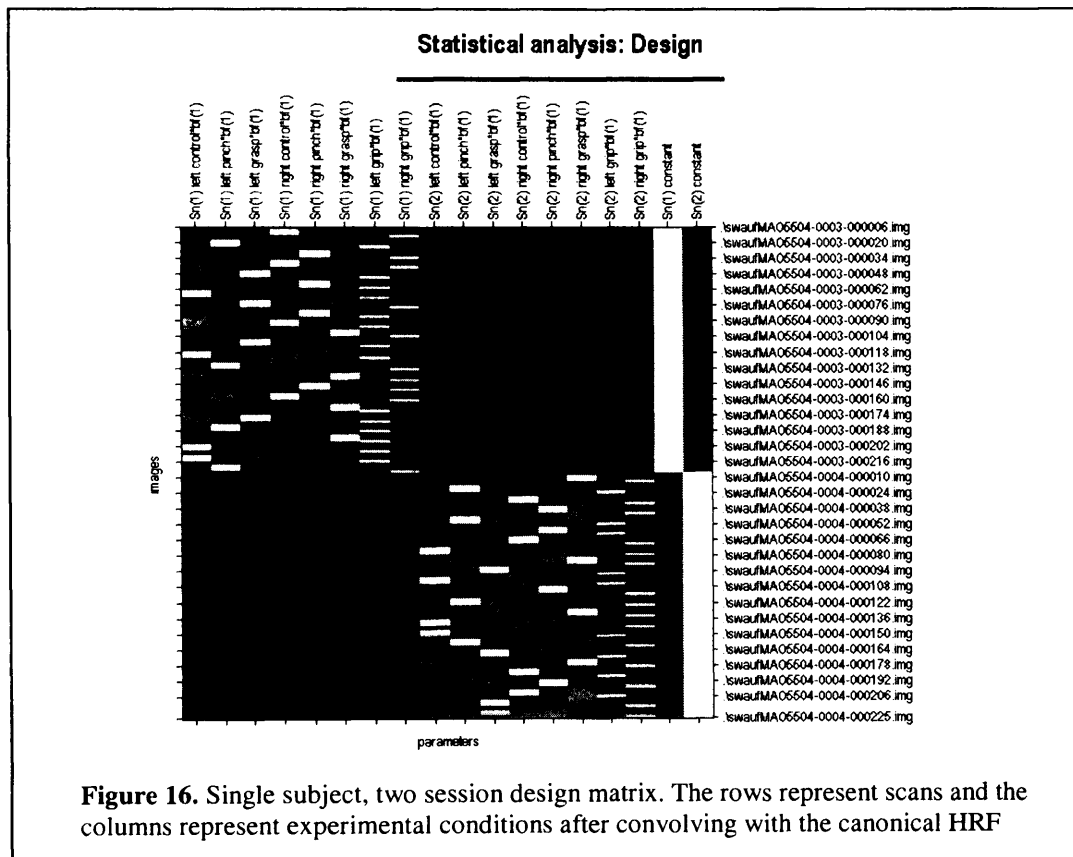
**1 form for Patient, 1 to be kept as part of the study documentation, 1 to be kept with hospital notes**

## Appendix F: Procedures for Statistical Analysis of fMRI Data

Statistical analysis was performed in three stages. In the first stage, data from single subjects were analysed separately using a single subject two-session fixed effects model. Each type of video was defined as a single covariate and modelled using a boxcar basis function. All hand grips (used to respond to the post-video question) were defined as a single event type and modelled as delta functions. All covariates were convolved with a canonical synthetic haemodynamic response function (HRF) and used in a general linear model (Friston, Holmes et al., 1995) together with a covariate representing the mean (constant) term over scans for each session (Figure 16).

The canonical HRF represents a typical BOLD response derived from a principal component analysis of data reported by Friston et al. (1998). Thus for each subject, voxel-wise parameter estimates for each covariate resulting from the least mean squares fit of the model to the data were calculated. The parameter estimates (or *betas*) for each covariate reflect the magnitude of increase in the BOLD signal during each condition. The statistical parametric maps of the t statistic ( $SPM\{t\}$ ) resulting from linear contrasts of each covariate (Friston, Holmes et al., 1995) were generated and stored as separate images for each subject.

The data for the second stage of analysis comprised the pooled parameter estimates for each covariate across all subjects. Contrast images for each subject were entered into a one sample t-test for each covariate of interest. The  $SPM\{t\}$ s were thresholded at  $p < .05$ , corrected for multiple comparisons across whole brain.



Further analyses were performed in order to determine (i) the influence of age on brain activity during each task, and (ii) regional correlations between brain activity and accuracy in the one-back task, using simple linear regression within SPM5. Here, the two orthogonal covariates were: (i) contrast images for each subject for the effect of interest and (ii) a single value representing the covariate of interest, e.g. age, for each subject (mean corrected and normalized across the group). SPM{t}s representing brain regions in which there is a linear relationship between the relevant parameter estimates and age were generated. The height threshold was set at  $p < .001$ , uncorrected, for multiple comparisons across whole brain, and the extent (or cluster) threshold set at  $p < .05$ , corrected for multiple comparisons across whole brain. For significant voxels the correlation coefficient for the plot of parameter

estimate against the covariate of interest was also calculated to illustrate the relationship.

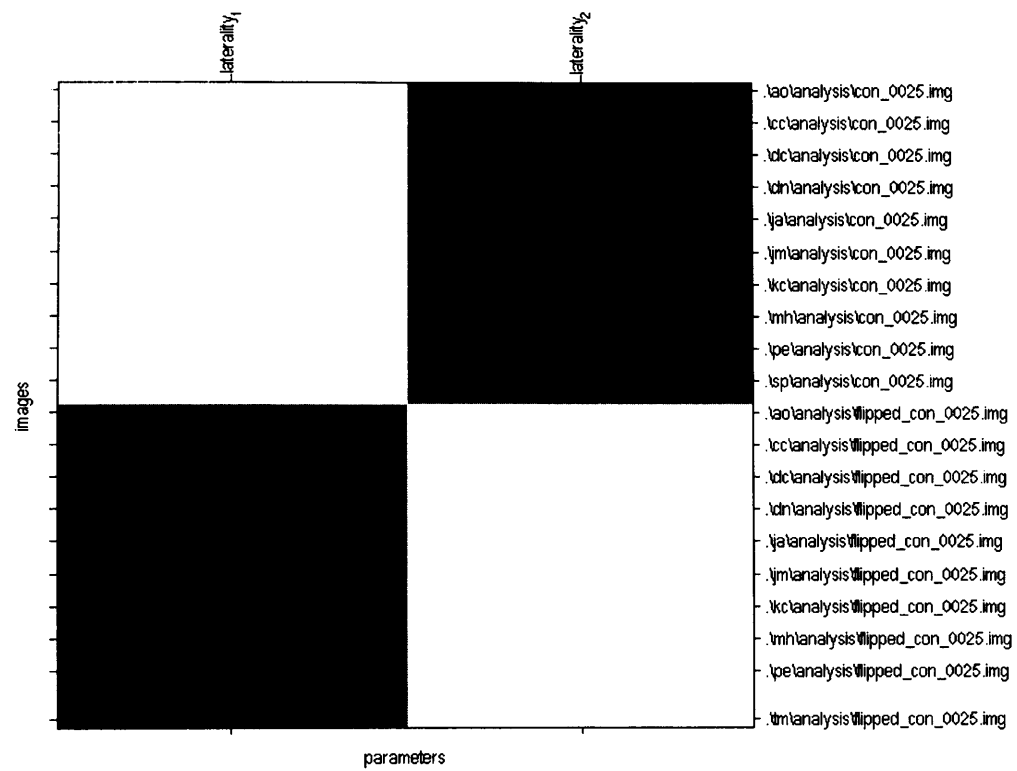
Analysis of single patient data conformed to the scheme described above. Single patients were compared to the control group, both individually and as a group, using 2-sample t-tests in SPM5. In this analysis, the brains of patients with right sided lesions were flipped about the mid-sagittal line, so that we could then assume that all patients had left sided lesions leading to weakness of the right (affected) hand.

All  $SPM\{t\}$ s were transformed to the unit normal Z-distribution to create a statistical parametric map ( $SPM\{Z\}$ ). All t-tests carried out within SPM were one tailed. Anatomical identification was carefully performed by superimposing the maxima of activation foci both on the MNI brain and on the normalised structural images of each subject, and labelling with the aid of the atlas of Duvernoy (Duvernoy, 1991).

### **Laterality analysis**

To address the question of whether activations were truly unilateral or bilateral within the control group, contrast images for the main effects of observing a hand in an active task were flipped about the sagittal plane (producing mirror images). Both unflipped and flipped contrast images were used in a factorial design (with ‘unflipped/flipped’ as the only factor (with two levels) (Figure 17). Truly unilateral activity was then determined by comparing unflipped versus flipped images. Truly bilateral activity was determined by a conjunction of flipped with unflipped images.





**Figure 17.** Design matrix for laterality analysis