THE FUNCTIONAL ANATOMY OF CEREBRAL REORGANISATION IN THE HUMAN SENSORIMOTOR SYSTEM

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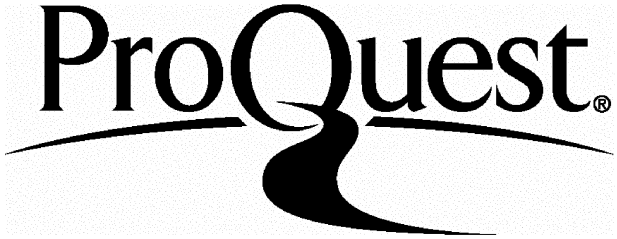

For Kate, Felix, and Joe

Abstract

Functional imaging techniques allow assessment of distributed regional brain activation in humans during the performance of specified tasks. To date, studies of the motor system in previously hemiparetic stroke patients have demonstrated task related brain activation over and above control subjects in a number of brain regions usually only recruited during more complex motor tasks. However, as most of these studies were performed in patients with good recovery, the relationship between these findings and the recovery process remained unclear. The purpose of the experiments in this thesis was to establish whether such a relationship exists by using functional magnetic resonance imaging (fMRI).

All experiments employed an isometric dynamic hand grip task (with visual feedback of the force exerted). Hand grip may be usefully performed by stroke patients with poor motor function. In the first experiment, I characterised the normal functional anatomy of hand grip in 26 control subjects. The task activated regions within the recognised motor network, and in a putative human 'grasping network', involving rostral ventral premotor cortex (Brodmann area 44) and intraparietal sulcus.

I was then able to make comparisons between task-related activations in chronic stroke patients with a wide range of outcomes. By setting the target force as a proportion of each patient's maximum grip strength, I was able to equalise effort exerted across patients. Outcome was assessed using nine outcome measures, and a single 'relative' score was calculated for each patient using a principal component analysis. This approach revealed that patients with poorer outcome activated a number of brain regions over and above those seen in the normal population. Patients with good outcome were less likely to do so. More formally, there was a negative linear correlation between task related activation in these regions and outcome.

In order to observe the evolution of motor activation patterns as a function of recovery, I performed longitudinal studies using the same motor paradigm over the first 8-12 months following stroke. In all patients, there was a negative

correlation between recovery and task-related recruitment of several brain regions, mainly within the distributed motor system. Furthermore, I was able to demonstrate a negative linear correlation between initial severity (at 10-14 days post stroke) and task related activation in the same regions.

These studies demonstrate a clear relationship between the degree of recovery and task related activation (both within and across subjects) in regions such as premotor cortex, supplementary motor areas, posterior parietal cortex and cerebellum, as well as in both ipsilesional and contralesional primary motor cortex (M1). All of these regions are involved in neural circuits with projections to both the motor output part of spinal cord, as well as to M1 itself. The increased activity may represent an alternative but less efficient means of generating motor output. A reduction in activation over time suggests that increasing efficiency in neural pathways and networks in these regions underlies the improvement in performance of a simple motor task, similar to the results of motor learning studies.

Lastly, by studying the same motor task across a group of normal subjects with a wide variety of ages, I was able to demonstrate that normal older subjects were also more likely to activate a more widespread motor network, in order to maintain performance. This finding is of significance, when considering that work in animal models suggests that the capacity for adaptive change is finite. There is a need therefore to establish an empirical understanding of how the brain responds to injury in relationship to recovery, and how other parameters, in particular age, have an effect on this response.

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PUBLICATIONS ARISING FROM WORK IN THIS THESIS

Published Papers

Ward NS, Frackowiak RSJ. Age related changes in the neural correlates of motor performance. *Brain* 2003; 126: 873-888.

Ward NS, Brown MM, Thompson AJ, Frackowiak RSJ. Neural correlates of outcome after stroke: a cross-sectional fMRI study. *Brain* 2003; 126: 1430-1448. Brain Advance Access published April 8, 2003: 10.1093/brain/awg145.

Ward NS, Brown MM, Thompson AJ, Frackowiak RSJ. Neural correlates of motor recovery after stroke: a longitudinal fMRI study. *Brain* 2003; 126: 2476-2496. Brain Advance Access published August 22, 2003: 10.1093/brain/awg245.

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Ward NS, Rowe JB, Frackowiak RSJ. Characterisation of the effects of different rate and force on the BOLD signal during hand grip. 7th Annual Meeting of the Organization for Human Brain Mapping, Brighton, UK, 2001.

Ward NS, Frackowiak RSJ. Age effects in a handgrip task using dominant or non-dominant hands: a functional MRI study. 8th Annual Meeting of the Organization for Human Brain Mapping, Sendai, Japan, 2002.

Ward NS, Brown MM, Thompson AJ, Frackowiak RSJ. Functional correlates of recovery from stroke in individual patients: a cross sectional fMRI study. 8th Annual Meeting of the Organization for Human Brain Mapping, Sendai, Japan, 2002.

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Ward NS, Thompson AJ, Brown MM, Frackowiak RSJ. The relationship between motor related brain activations and recovery after stroke: a cross sectional fMRI study. 12th European Stroke Conference, Valencia, Spain, 2003.

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Chapter 1

NEURONAL PLASTICITY: THE KEY TO STROKE RECOVERY?

1.1 Introduction

Patients suffering from a stroke often undergo some degree of functional recovery (Twitchell, 1951). What is remarkable, but often taken for granted is that this recovery may occur over weeks and months, and can be influenced by external manipulations, including environmental, pharmacological and psychological. The extent of this recovery can up to a point be predicted on the basis of parameters such as lesion size, premorbid health etc. However, significant variation occurs, implying that outcome is influenced by other variables. It is the responsibility of the clinician to attempt to influence these variables in order to facilitate the best possible outcome for each patient. To date the cornerstone of restorative neurology in the field of stroke medicine has been good general medical care together with more specific therapy (physiotherapy, occupational therapy, speech and language therapy). This is of course still the case. However clinicians are

becoming aware of advances made in the basic sciences, which will add a new dimension to the treatment of functional deficits in patients suffering single insult brain injury.

This chapter will discuss the concept of plasticity in the brain, how brain damage interacts with the mechanisms underlying plasticity, and how both environmental and pharmacological manipulations can improve outcome by influencing these processes. It will be seen that most of this work has been performed in animal models of focal brain injury. It is this body of work that provides the inspiration to those seeking to investigate the same processes in the working human brain, in the belief that functional outcome after stroke can be further enhanced by manipulating the process of plasticity.

1.2 Early recovery: the principles of acute stroke management

In the first few minutes and hours after stroke, physicians take an active approach to management with the aim of preserving as much brain tissue as possible (Brown, 2002). Thrombolytic therapy using tissue plasminogen activator is beneficial in acute ischaemic stroke, but only when given within 3 hours of stroke to suitable patients in specialised units (Lees, 2000). Many neuroprotective drugs have been tested successfully in animal models of stroke, but none has proved effective in randomised trials in man to date. However, other non-pharmacological strategies are often employed to protect the brain from further ischaemia and extension of infarction. For example, systolic blood pressure can be maintained within a given range, antipyretic drugs given for fever and blood glucose maintained within the normal range. Nevertheless, despite these measures many

patients with stroke continue to have significant disability after the acute period. Hence, enhancing recovery using modern rehabilitation techniques continues to play an essential part in stroke treatment.

1.3 Late recovery: the principles of the multidisciplinary approach

Randomised clinical trials have provided good evidence that the best way to enhance recovery is to admit the patient to a stroke unit (Stroke Unit Trialists' Collaboration, 2000). These benefits are achieved not simply by gathering the patients together in a single location labeled "stroke unit", but by the development of a specialised multidisciplinary team. How much each component contributes to the success of the stroke unit to its success is not entirely clear, but recent evidence supports the effectiveness of physiotherapy for stroke patients in the post acute phase. A meta-analysis combining results of seven randomized controlled trials on the effects of different intensities of therapy after stroke reported small but significant reductions in mortality and significant improvements in activities of daily living (ADL) scores as a result of higher intensity therapy (Langhorne *et al.*, 1995, 1996). Furthermore, Kwakkel *et al.*, (1997) examined nine trials of physiotherapy in stroke patients involving 1051 patients, and concluded that there was a small but significant correlation between intensity of therapy and outcome. In support of the notion that targeted therapy has a specific rather than non-specific effect, greater intensity of leg rehabilitation improves functional recovery and health-related functional status. Conversely, greater intensity of arm rehabilitation results in small improvements in hand dexterity (Kwakkel *et al.*, 1999). This result suggests that failure to demonstrate a benefit with

physiotherapy in the past might be related to the fact that assessments were often made using global ADL scales, rather than parameters relating directly to the form of training being given. For example, a study involving 148 hemiparetic patients undergoing an intensive period of training, demonstrated that gait symmetry (a principle goal of many physiotherapy programs) was unaffected. However, specific measurements of stance duration, weight acceptance, and push off of both legs improved significantly (Hesse *et al.,* 1994). Furthermore recovery of gait function in the severely hemiplegic patient using treadmill training, functional electrical stimulation, individually or in combination with each other, have been demonstrated to improve specific aspects of gait, indicating a significant task specific training effect (Hesse *et al.,* 1995).

It might be argued that the only truly relevant measurements of outcome or recovery are global functional assessments in the home environment. If however, one wishes to investigate the mechanisms of recovery, it is important to document the induced changes as carefully and in as detailed a fashion as possible. For example, although patients may regain the ability to solve simple spatial motor problems and perform well on ADL tasks, they may still exhibit reduced skillfulness of an affected hand (Platz *et al.,* 1994). Although it is essential to know whether a patient becomes independent, it is also of importance to know, for example, what effect the motor and sensory experience of physiotherapy has on the motor system. These are two different questions, the latter being concerned with the relationship between changes in the nervous system and consequent functional recovery. To begin to understand mechanisms of recovery in these patients, and potentially to be able to manipulate these mechanisms, one must look

beyond using purely ADL scores as an assessment. This is an important lesson in assessing any form of rehabilitative strategy if we are to avoid discarding potentially useful treatments as a result of selecting poor or inappropriate outcome measures.

1.4 Plasticity in the normal brain

The term 'plasticity' is often used when describing putative mechanisms of recovery after focal brain injury, but it is an ill-defined term. Over fifty years ago, Hebb observed that rats raised in his home had an increased capacity to learn compared to laboratory-raised rats. In an attempt to explain this phenomenon he postulated that during learning, increments in synaptic efficacy occur when firing of one neuron repeatedly produces firing in another neuron to which it is connected (Hebb, 1947; Hebb 1949). This encapsulates the notion of plasticity as a change in behaviour (i.e. learning) associated with a change of function at the level of the synapse. Expressed in a more general framework, a definition of plasticity might specify a change in brain structure over time with a consequent change in function. The cortex, with its myriad synaptic connections is the ideal site for this plasticity to take place (Sanes and Donoghue, 2000). These plastic changes may occur in a number of ways. Firstly, it has been repeatedly demonstrated that exposure to enriched environments and motor learning in adult animals is associated with morphological change in the cortex. In particular, growth of dendrites, synaptogenesis, astrocytic proliferation, and even structural changes at the level of the pre- and post-synaptic membranes have all been observed (Kolb, 1995; Ivanco and Greenough, 2000; Bury *et al.*, 2000). Secondly,

echoing the sentiments of Hebb, long term potentiation (LTP) and long term depression (LTD) have long been known about as mechanisms of changing synaptic efficacy in the context of learning studies, particularly in the hippocampus (Collingridge and Bliss, 1995). More recently there is evidence that these processes can occur in neocortex if certain conditions, such as a concurrent ascending input, are in place (Hess *et al.,* 1996). Motor skill learning is accompanied by changes in the strength of connections within primary motor cortex in animal models (Rioult-Pedotti *et al.,* 1998), presumably by LTP-like mechanisms. Thirdly, the influence of one cortical neuron on another can be altered by factors other than external environment or practice. Jacobs and Donoghue (1991) performed an experiment in which bicuculline (a GABA antagonist) was applied to forelimb area of rat motor cortex. Stimulation of the adjacent cortex (normally representing the vibrissae) then led to forelimb movements. This suggests that cortical maps are maintained at least in part by GABA, and can be altered by pharmacological manipulation.

In studying the human brain there are clearly greater limitations. Very different techniques are required to study alterations in cortical function and neural networks. Studies using transcranial magnetic stimulation (TMS) have demonstrated changes in cortical function in response to sensory input (Hamdy *et al.,* 1998), motor imagery (Hashimoto and Rothwell, 1999), and motor practice (Pascual-Leone *et al.,* 2000). Functional imaging has been used to demonstrate changes in the organisation of neural networks during motor learning (Toni *et al.,* 1998; Karni *et al.,* 1995).

Work in both animals and humans suggest that the brain is not hardwired. The notion that the brain, and in particular the cortex, has the capacity to change structure and consequently function is now widely accepted. Up to now I have discussed the potential for plastic change in the normal brain, but I will now consider whether plasticity also occurs in the damaged brain, and whether this process in any way contributes to functional recovery.

1.5 Plasticity in the lesioned brain

A key message that has emerged over the last few years is that the lesioned brain and the normal brain are different when it comes to the potential for plastic change. There is much evidence now to support the idea that the lesioned brain has an increased capacity for plastic change. Developmental proteins not normally expressed in the adult brain re-emerge in the hours and days following focal brain injury. These proteins are involved in changes in the extra-cellular matrix, structure of glial support cells, neuronal growth, apoptosis, angiogenesis and cellular differentiation (Cramer and Chopp, 2001). Recent work has demonstrated that following ischaemic damage in adult rats, new progenitor neurons proliferate and migrate from the subventricular zone and dentate gyrus to the site of damage. Here, these new cells express morphological characteristics of the recently damaged cells (Arvidsson *et al.,* 2002). Structural changes have also been observed, with evidence of neurogenesis (Gould *et al.,* 1999), increased dendritic branching (Jones and Schallert, 1992), and synaptogenesis (Jones *et al.*, 1996). The correlation between some of these changes and behavioural recovery becomes clearer when considering that the magnitude and temporal course of cellular

events often parallels this recovery (Cramer and Chopp, 2001). It is also interesting to consider the spatial distribution of such changes, with synaptogenesis (Stroemer *et al.,* 1998) and axonal outgrowth (Kawamata *et al.*, 1998) seen in perilesional tissue in rats, and evidence of dendritic branching in homotopic cortex in the non-lesioned hemisphere (Kozlowski *et al.*, 1996). The changes in cortical structure and function that might mediate recovery may therefore occur at sites distant from the lesion. The idea that intact areas of the brain become functionally and metabolically inactive because they are disconnected from the site of focal lesions (a phenomenon known as diaschisis), and that this might have an impact on not only the clinical presentation but also on recovery, was first discussed at the beginning of the last century by Von Monakow (Von Monakow, 1914). However, other forms of metabolic change distant from the site of the lesion have also been observed in animal models. Widespread areas of hyperexcitability in cortex, distinct from the areas of metabolic depression, have been observed in several studies, both ipsilateral and contralateral to a lesion (Witte and Stoll, 1997). In animal models at least, the extent and time course of recovery of these areas of hyperexcitability does not correlate with the changes in metabolic diaschisis, so they are presumably distinct processes. It has been proposed that it is these areas of hyperexcitability that develop in the first five days and partially reverse over months that may be the substrate for use dependent plasticity (Witte, 1998). Hagemann *et al.,* (1998) demonstrated areas of hyperexcitability in the surround of focal cortical infarcts in rat brains. These areas were associated with reduced GABAergic inhibition and increased NMDA receptor expression. Furthermore, they demonstrated that the

induction of long term potentiation (LTP) was facilitated in these areas. Thus, in this hyperexcitable cortex, inputs from neighbouring cortical neurones may become more efficacious, shifting cortical representations. LTP is an activity driven process, and it is tempting to think that motor training is what provides this crucial input. That this is the mechanism of recovery of behavioural function is not established, but the link is tantalising.

In human studies using different techniques, there is also evidence of changes in the organisation of cortical networks following focal brain lesions. Enlargement of cortical motor maps demonstrated with TMS correlates with functional improvement in stroke patients (Traversa *et al.*, 1997), and changes in activation maps of recovered patients post stroke have been demonstrated in both motor (Weiller *et al.,* 1993, Cramer *et al.,* 1997), and language studies (Warburton *et al.,* 1999), as will be discussed in chapter 2. However, there is as yet no direct evidence to suggest that the lesioned human brain has an increased capacity for plastic change compared to the unlesioned brain. Furthermore, many of the animal models mentioned are models of cortical damage, and it is not clear that these data are relevant to subcortical stroke. However, the data that are available from animal models encourages us to speculate that we may be able to take advantage of changes in the human lesioned brain in order to promote functional recovery.

1.6 Promoting functional recovery after focal cerebral damage

1.6.1 Environmental manipulation

The brain has the capacity for plastic change, and that this seems to increase in the lesioned brain as a consequence of expression of trophic factors and other changes previously discussed. How might we take advantage of such changes to influence outcome? A number of studies have demonstrated that these changes are dependent not only on the lesion, but on experiential demand (Schallert *et al.*, 2000). For example, in rats subjected to unilateral sensorimotor cortex damage, restraining of the impaired forelimb leads to reduced dendritic arborization in surrounding cortical tissue. This is not seen if the impaired limb continues to be used (Jones and Schallert, 1992). Animal studies have been supportive of the idea of task specific training effects in cortically injured subjects, and have begun to shed light on the underlying mechanisms. Nudo *et al.*, (1996), in an important study, trained Squirrel monkeys in the execution of a complex motor task using a hand. Focal infarcts of a small portion of the hand representation in cortex were induced and five days later intensive retraining identical to that previously applied was undertaken. This continued until pre-infarct levels of performance were attained. Using intracortical microstimulation techniques, they found that spared hand areas had either been preserved or had expanded into regions previously occupied by elbow and shoulder representations. Animals in whom retraining had not been attempted and in whom recovery had been less marked had lost remaining hand representation in the cortex. Rehabilitative training would therefore seem to have had an effect on reorganization of intact cortex, as measured by changes in cortical maps, with a consequent beneficial effect on

motor recovery. Exposure of animals to an enriched environment enhances dendritic growth and synapse formation (Schallert *et al.*, 2000), and has also been demonstrated to enhance post brain injury recovery (Ohlsson and Johansson, 1995). This effect is likely to be related to experience and consequent cognitive processing, as physical exercise on its own does not produce such significant results on post injury motor recovery (Gentile *et al.*, 1987).

Parallels certainly exist in human studies, as has already been mentioned. In particular, improvements in motor performance in the chronic setting have been demonstrated with constraint induced therapy (CIT), based on overcoming learned non-use of the affected limb (Taub, 1993), and have been accompanied by increases in cortical representation of the affected limb (Liepert *et al.*, 2000). Other theoretically derived techniques, such as bilateral arm training are similarly under investigation (Whitall *et al.*, 2000). Underpinning all of these techniques is the concept of activity driven change, the notion that by increasing the activity of neurons in strategically located cortical regions, structural change will ensue, resulting in improvement of function. A striking example is that of an increase in pinch grip strength induced by 2 hours of median nerve stimulation in hemiparetic patients (Conforto *et al.*, 2002). This notion is useful yet likely to be oversimplified. Many aspects of language and motor function in the normal brain are still under active investigation. Motor learning for example is the product of complex dynamic interactions between cortical and subcortical structures that is likely to be optimized by certain conditions and learning techniques (Hikosaka *et al.*, 2002). Until we have an empirical understanding of these optimal parameters

in both health and disease, our attempts at promotion of functional recovery will not have the well grounded theoretical basis that is required for progress.

A critical issue concerns the timing of interventions. The evidence presented seems to suggest that many of the changes that take place after stroke, the very changes that we may need to take advantage of to promote functional change, occur early after the lesion. It would seem therefore, that to take full advantage of these changes, intervention must occur relatively soon after stroke. There has however, been a reluctance to use many interventions such as forced therapy approaches in the acute setting because of evidence that such an approach could lead to exacerbation of cortical damage (Kozlowski *et al.,* 1996). This overuse-dependent exaggeration of injury can be blocked by administering MK-801 (NMDA antagonist), suggesting that glutaminergic mechanisms are involved. The question of timing of an intervention is it appears critical, because there are definite changes in the molecular and cellular environment at a certain time point after injury. For example, it has been hypothesized that during early development new synapses with high NMDA:AMPA receptor ratios are formed. This ratio rapidly becomes similar to that seen in adults. In any period where new synapses are forming, such as early development, new learning, or post focal brain damage (Stroemer *et al.,* 1998), use dependent plasticity is likely to occur in the new synapses with high NMDA:AMPA receptor ratios, which may explain why NMDA antagonists, although thought to be protective in acute ischaemia, may slow or prevent plastic changes occurring in perilesional tissue, with subsequent impairment of functional recovery (Barth *et al.,* 1990). Understanding of these processes is crucial if we are to use interventions correctly.

1.6.2 Pharmacological manipulation

There has been recent interest in whether the processes collectively described as neuronal or synaptic plasticity can be influenced by pharmacological manipulation. As early as 1942, investigators concluded that the cholinergic drugs, strychnine and thiamine could enhance the rate and degree of recovery from motor cortex damage in monkeys (Ward and Kennard, 1942). However, by the 1950's it was felt that pharmacological stimulation of the reticular activating system was the key to facilitating recovery. Amphetamine was first used in 1946 by Maling and Acheson (1946), who demonstrated that this drug transiently restored righting reflexes in low decerebrate cats, and subsequently Meyer *et al.*, (1963) temporarily restored the placing reflex in decorticate cats by the administration of amphetamine one year after they had originally undergone surgery. Faugier-Grimaud *et al.*, (1978) also demonstrated that a deficit could be temporarily reinduced after recovery had taken place. They performed either unilateral or bilateral parietal lesions in Java monkeys, inducing deficits in visually guided reaching tasks, after which spontaneous recovery took two weeks. One year after surgery the monkeys were given a small dose of the general anaesthetic, ketamine, which resulted in an immediate but temporary return of the deficit, with the deficit being unilateral or bilateral depending on the number of lesions performed one year previously.

Feeney and co-workers revisited amphetamine as a possible neuromodulator of functional recovery (Feeney *et al.*, 1982). A series of experiments were carried out on rats that had undergone suction ablation of the sensorimotor cortex. Amphetamine given after ablation, and practice both appeared to speed up

recovery significantly, but crucially practice contributed to this effect only in the context of amphetamine administration, and vice versa. Haloperidol given with amphetamine blocked the effect, and given alone retarded recovery, suggesting a role for dopamine (DA) neurotransmission. Further observations implicated the cerebellum in this effect. Firstly, both amphetamine and haloperidol worsen beam walking recovery in rats with cerebellar injury (Boyeson and Feeney, 1991), and secondly microinfusions of noradrenaline (NA) into the cerebellum contralateral but not ipsilateral to the site of a cortical injury, mimics the systemic effects of drugs on beam walking recovery in rats (Boyeson and Krobert, 1992).

Although the effect of haloperidol in Feeney's initial experiments suggested that the effect was mediated by DA, further evidence implicates NA. Lesions to the contralateral but not ipsilateral dorsal noradrenergic bundle (projecting from the locus coeruleus to the cerebral cortex), impair motor recovery after subsequent cortical lesion (Goldstein and Bullman, 1997). Alpha-2 antagonists (increasing noradrenergic effect) such as yohimbine and idazoxan, have also been found to facilitate motor recovery in a single dose (Sutton and Feeney, 1992). Conversely drugs that decrease NA release in the central nervous system, or block post-synaptic effects (i.e. alpha-1 antagonists/alpha-2 agonists), are likely to be harmful to recovery in the above model, and in fact clonidine (alpha-2 agonist) (Goldstein and Davis, 1990) impairs beam walking recovery, in the same way as haloperidol. In an echo of the work done by Faugier-Grimaud *et al.* in 1978, it was noted that these drugs not only impair recovery, but if given to a rat that has made a spontaneous recovery, will reinstate the deficit temporarily, to a degree

proportional to the original deficit. This effect has been seen with several drugs (clonidine, prazosin, phenoxybenzamine) and across species (Feeney, 1997).

Speculation as to the mechanism of action of these effects is fascinating. Noradrenergic induced changes in local metabolism or enhancement of LTP may allow motor experience to induce permanent changes. We have already discussed the evidence pointing towards how this may occur in relation to LTP (Hagemann *et al.*, 1998). It is also fascinating to note that the effects of neurotransmitters (and drugs that affect these neurotransmitters) on LTP induction, correlates strongly with their effects on recovery of function after sensorimotor cortex injury in animals (Goldstein, 1990). More recently Stroemer and co-workers (1998) induced unilateral cortical ischaemia in a group of rats, and then treated one group with amphetamine and one with saline. Levels of GAP-43 and synaptophysin, as markers of neurite growth and synaptogenesis respectively, were measured in peri-infarct tissue at different intervals after the lesion was induced. Behavioral recovery was measured at the same time intervals. Levels of GAP-43 and synaptophysin were found to be significantly elevated and the degree of elevation correlated with behavioral recovery in a temporal fashion. It is tempting therefore to suggest that the increase in expression of these proteins promotes structural change (as the possible substrate of recovery) directly, but it is known that these proteins are associated also with release of NA and DA (Dekker *et al.*, 1989), and possibly with LTP (Iwata *et al.*, 1997), both of which may be important factors themselves in functional recovery.

The fact that the recovery of function is so susceptible to reversal by pharmacological agents (e.g. ketamine, prazosin, clonidine), suggests that changes

are not purely anatomical and that a change in the neurochemical balance of interacting systems has been effected. Both Luria (1963) and Meyer (1972) have suggested that lesions in these animal models cause a suppression of retrieval of motor engrams, which have been formed in the brain as a result of previous learning and experience. Perhaps these motor engrams are not destroyed by cortical lesions but become inaccessible, and noradrenergic enhancement allows them to be accessed once more. This is of course speculation, but it is a hypothesis that addresses the problem of assuming that localized cortical changes can substitute for profound disturbances in distributed networks. Restoration of dynamic interactions between the nodes of these networks is likely to be crucial in regaining meaningful recovery. Indirect evidence to support this comes not only from systems neuroscience, but also from cellular and molecular studies. New neuronal cells migrating to the site of damage following middle cerebral artery occlusion in rats, express the morphological characteristics of neurons which form part of the basal ganglia-cortical loops, so important in motor control (Arvidsson *et al.*, 2002).

There is also evidence that modulation of other neurotransmitter systems can have similar effects. Dopamine has already been mentioned in relation to Feeney's work with haloperidol. Apomorphine reduces the severity of experimentally induced neglect from prefrontal injury, and spiroperidol reinstates this neglect (Feeney, 1997). GABA infused intracortically impairs beam walking recovery in rats (Brailowsky *et al.*, 1986), and diazepam impairs recovery of sensory asymmetry caused by unilateral damage to the anteromedial cortex in rats (Schallert *et al.*, 1986). Acetylcholine antagonist scopolamine interferes with

recovery after motor cortex infarction in rats (De Ryck *et al.,* 1990), and in monkeys, cholinergic drugs increase the rate of recovery in animals with motor cortex lesions (Ward and Kennard, 1942). NMDA antagonist MK-801, has been found to be detrimental if given during the recovery period in rats (Barth *et al.,* 1990), which is in contrast to its proposed neuroprotective effect if given immediately after an infarct.

More recently, a greater understanding of the molecular and cellular events occurring post injury, has lead to attempts to manipulate them for the purposes of promoting functional recovery. Candidate compounds include osteogenic protein-1 (Ren *et al.,* 2000), brain-derived neurotrophic factor (Rossi *et al.*, 1999), fibroblast growth factor-2 (Kawamata *et al.,* 1997) and stem cell treatment (Kolb *et al.,* 1998a).

In summary therefore, experiments in animals point most strongly towards the influence of neurotransmitter systems on functional recovery after focal brain injury. The effect is dose dependent, and the timing of administration is crucial, with the effect being dependent on close temporal linkage to behavioral experience.

What of attempts to translate these findings into promotion of functional recovery in humans? Much of the early work in this field has been done by Alexander Luria and colleagues in soldiers with head injuries sustained during the Second World War (Luria, 1963; Luria *et al.,* 1963). They proposed two types of functional disturbance as a result of focal brain lesions. Firstly cell death, and secondly functional inhibition of intact neurons. They suggested that patients in whom the latter predominated might benefit from "removal of the diaschisis, restoration of

synaptic conduction or to use another term, de-blocking" (Luria *et al.,* 1963). It was proposed that this could be achieved by the combination of two approaches. Firstly the administration of a pharmacological agent "capable of removing inhibition, modifying mediator metabolism, and restoring disturbed synaptic conduction" (Luria *et al.,* 1963), and secondly by methods of training which promote 'de-blocking', the essence of which is "that by means of various methods the level of excitability in certain functional systems is raised and the corresponding functions are 'de-inhibited'" (Luria *et al.,* 1963). The main de-blocking agents used by these investigators were anticholinesterases. In one such experiment, neostigmine was administered to a patient with a non penetrating wound of the premotor area. Rhythmic tapping movements were recorded before and after neostigmine was given, and improvements of 'dynamic co-ordination' were noted after the drug, when none had been obtained with repeated training attempts prior to this experiment (Luria, 1963). Luria also made claims that the rate of recovery from aphasia could be increased, as long as the lesion was not in what he described as the 'primary speech areas', using galantamine, (a specific, competitive and reversible acetylcholinesterase inhibitor now under investigation for the treatment of dementia) (Luria, 1963).

It is surprising that despite this large body of work, further studies of pharmacological enhancement of recovery were not pursued further until nearly 40 years after Luria's original work was published. The first trial of noradrenergic enhancement coupled with physical therapy in human subjects was published in 1988 (Crisostomo *et al.,* 1988). In this experiment patients who had suffered from hemiplegic stroke were randomized to receive either 10 mg amphetamine or

placebo, 45 minutes prior to physiotherapy. Follow up assessment 24 hours after treatment indicated a 40% improvement from baseline scores compared to placebo. Small numbers of patients in this study (eight in total), make interpretation difficult, as the authors stated. A subsequent failure to replicate these findings by Borucki *et al.* (1992) were attributed to different experimental design, in particular a failure to schedule physiotherapy immediately after amphetamine treatment. Perhaps it was also significant, in view of the possible therapeutic window, that treatment was not started until over a month after the stroke. More recent studies with d-amphetamine have shown conflicting results (Walker-Batson *et al.*, 1995; Sonde *et al.*, 2001), but positive results have been published for daily doses of l-dopa (100mg) used in conjunction with physiotherapy (Scheidtmann *et al.*, 2001). Studies have initially focussed on motor recovery, most likely because of obvious parallels with animal studies, but similar studies looking at recovery in aphasia after stroke have been performed (Walker-Batson *et al.*, 2001).

Thus exogenously administered drugs may alter the balance of extracellular concentrations of various neurotransmitters, which might have an effect on functional outcome following focal brain injury. Goldstein *et al.* (1990) performed a retrospective analysis of the effect of drugs, (predicted from animal models to have a deleterious effect on motor outcome following focal brain injury) on outcome in stroke patients. Patients receiving phenytoin, benzodiazepines, or alpha-adrenergic antihypertensives at the time of stroke or shortly afterwards had poorer outcomes than controls who did not receive any of these drugs. This was found to be the case for a number of outcome measures, covering both activities

of daily living and specific motor function, and 30 day recovery rates. These findings were replicated by the Acute Stroke Study Investigators (Goldstein, 1995), using a group of patients who were themselves the control group in a prospective acute interventional trial. 40% of these patients received one or a combination of drugs predicted to impair recovery, and were found to have poorer recovery as measured by a variety of measures. Similar findings have been published relating to the adverse effects of certain antihypertensives (Porch *et al.*, 1986). These analyses were retrospective and could not exclude the possibility that patients were given these drugs for medical reasons that themselves would predict poorer outcome, but this area of research clearly warrants further investigation.

Evidence therefore exists that certain drugs influence behavioral recovery in humans following focal brain injury. This conclusion has implications in as much as it suggests that more could be done to enhance recovery in patients and that certain drugs are probably best avoided after stroke.

1.7 Functional recovery: the influence of age

The capacity for plastic change in any brain may be finite, particularly in older subjects. Functional imaging studies have examined for differences in recruitment of brain regions during both motor (Mattay *et al.*, 2002) and cognitive tasks (D'Esposito *et al.*, 1999), and many of these studies have found greater activations in older subjects in a number of regions compared to younger subjects. However, this may only be the case for those elder subjects in whom level of performance is comparable to that in the younger subjects (Mattay *et al.*, 2002). It has been

suggested that interruption of the normal neural networks subserving cognitive performance by age related neurodegenerative and neurochemical changes underlies decline in function (Volkow *et al.*, 1998; Wenk *et al.*, 1989), but that compensatory processes in cortical and subcortical function allow maintenance of performance level in some people. We also know that after injury induced reorganisation of the brain, the capacity for subsequent adaptive change is reduced (Kolb *et al.*, 1998b). It is possible that the adaptive changes that have been observed in older brains and injured brains may in turn limit the capacity for further reorganisation after injury. This clearly has implications for what we can expect from therapeutic techniques designed to promote cerebral reorganisation after stroke in older subjects. A greater understanding of age related changes in the functional reorganisation of the brain will be crucial in unraveling the relationship between normal ageing and pathological processes.

1.8 Summary

Until recently it has been on the basis of intuition rather than evidence that physicians have recommended post-acute therapy for stroke patients. Evidence now exists that stroke units work, and that specific retraining techniques have measurable benefits. A wealth of evidence has been produced from work in animal models that the lesioned brain changes at a molecular, cellular and systems level, in a way that promotes experientially driven changes in synaptic structure and function. There is clearly a bias towards motor studies in animal models, but the principle of a post lesional plastic brain applies to any cortical function. Many questions remain unanswered, including whether the older human brain also has

this capacity for plastic change, and the challenge is now to advance our understanding of the science of recovery after brain damage in humans, and crucially how we can use this information to promote recovery.

Chapter 2

THE CEREBRAL BASIS OF FUNCTIONAL RECOVERY AFTER STROKE:

INSIGHTS FROM FUNCTIONAL IMAGING STUDIES

2.1 Introduction

The previous chapter introduced the idea of the plastic brain, and how changes in cerebral structure are related to changes in function. It is not readily possible to study this process at the cellular level in humans, but functional imaging techniques provide an opportunity to study changes at a systems level. The experiments described in this thesis investigate reorganisation within the motor system, and it is well understood that the motor system operates as a distributed network of brain regions, with dense interconnections. It is this form of distributed network that is so amenable to investigation with whole brain functional imaging. The investigation of cerebral reorganisation after focal brain injury in humans is less well advanced than similar work in animal models. However, it is clear that these approaches each have something to offer in the development of a

neurobiological framework underpinning our understanding of how recovery after focal brain injury might be enhanced.

I will now review the functional imaging data from studies concentrating on motor recovery in patients after stroke that lead up to and informed the design of the experiments described in chapters 6, 7, and 8. Studies performed in other laboratories that have been published since my own studies were undertaken will be discussed in the relevant chapters where appropriate.

The theoretical background to the techniques of single photon emission computerised tomography (SPECT), positron emission tomography (PET), and particularly functional magnetic resonance imaging (fMRI) are discussed in detail in chapter 3. In brief, all three techniques rely on the assumption that neuronal activity is closely coupled to a local increase in cerebral blood flow secondary to an increase in metabolism. SPECT and PET rely on mapping the distribution of radioactive tracers. Functional MRI includes many different methods, but the following studies refer to blood oxygen level dependent imaging (BOLD) techniques. During an increase in neuronal activation, there is an increase in local cerebral blood flow, but only a small proportion of the oxygen is used. There is therefore a net increase in the tissue concentration of oxyhaemoglobin and a net reduction in the tissue concentration of the paramagnetic deoxyhaemoglobin in the local capillary bed, and draining venules. The magnetic properties of haemoglobin depend on its level of oxygenation, so that this change results in an increase in signal intensity on T2*-weighted images.

2.2 Studies performed at rest

Attention has focused on task-related activation studies in patients with stroke. However, it is possible to measure areas of altered cerebral metabolism and blood flow at rest and look for correlations between the pattern of these changes and functional outcome. As already mentioned, the concept of diaschisis was first described by Von Monakow (1914) and termed diaschisis. In a human PET study, survival of the metabolically active cortex surrounding an infarct correlated with neurological recovery in acute stroke patients (Furlan *et al.*, 1996). The evidence regarding the role of the reversal of diaschisis (rather than recovery of stunned cells in the ischaemic penumbra) in functional recovery is however conflicting.

Studies using PET and SPECT have lead to several patterns of diaschisis being recognised, each depending on the site of the lesion (Baron, 1989).

(1) *Crossed cerebellar diaschisis* consists of a depression in metabolism and cerebral blood flow in the cerebellar hemisphere contralateral to a supratentorial lesion. It is likely to be the result of damage to the descending corticopontocerebellar connection, thus representing transneuronal functional depression (Baron *et al.*, 1981). The presence of crossed cerebellar diaschisis in the acute stage does not seem to be prognostic given its persistence during functional recovery (Infeld, 1995), whereas its absence predicts good motor outcome (Serrati *et al.*, 1994).

(2) *Ipsilateral thalamocortical diaschisis* describes the phenomenon of hypometabolism of the entire cortex ipsilateral to a thalamic or thalamocapsular infarct in patients studied some weeks or months after stroke (Baron *et al.*, 1986). The degree of hypometabolism seems to correlate with global neuropsychological

deficits, neglect, and aphasia (Baron *et al.*, 1986), but it is not clear whether this in itself contributes to poor late motor recovery. Subsequent improvement in the ratio of ipsilateral to contralateral metabolic rate correlates with subsequent cognitive improvement in the patients studied, but is not reliable in individual patients (Baron *et al.*, 1992).

(3) *Transhemispheric diaschisis.* In addition to the ipsilateral thalamocortical hypometabolism described above, a reduction in cortical metabolism has been observed in the contralesional hemisphere (Lenzi *et al.*, 1982; Baron *et al.*, 1992). This may be observed with both cortical and subcortical middle cerebral artery territory lesions and is thought to be due to transcallosal mechanisms. The literature concerning both early and late transhemispheric diaschisis in both humans and animals has been reviewed elsewhere (Andrews, 1991), but the relationship, if any to the functional outcome remains unclear. Since then a number of studies have been more interested in whether the observed changes have any prognostic implications, but with varying results.

Bowler *et al.,* (1995) performed SPECT scans in 50 unselected patients with cerebral infarcts at the time of infarct and 3 months later. The authors could not demonstrate that diaschisis independently added to the clinical deficit after stroke, and they found no correlation between recovery and reduction in diaschisis.

Di Piero *et al.*, (1992) used serial PET to perform a similar study, but examined for regional differences in cerebral metabolic rate of oxygen ($CMRO_2$) in the first week following stroke and again three months later. They found that the degree of recovery in motor function was related to the increase in $CMRO_2$ in intact cortical areas functionally connected to the site of the infarct. Recovery was least

pronounced in those with no increase in the relative cortical $CMRO_2$, intermediate in those with increases in cortical areas of the contralesional hemisphere only, and greatest in those with increases in both hemispheres.

Adopting a cross-sectional approach, Pantano *et al.*, (1996) measured rCBF in a number of patients 3 months after stroke and found that higher resting levels of rCBF in contralesional thalamus, basal ganglia and premotor cortex predicted subsequent functional improvement. Binkowski *et al.*, (1996) found that poorer outcome 4 weeks after stroke was associated with reduced cerebral metabolic rate of glucose (rCMRGlu) in the ipsilesional thalamus measured 2-4 weeks post stroke. It has been suggested that these patterns of diaschisis may reflect other parameters more closely related to prognosis, such as the size of the infarct, but this thalamic hypometabolism was related to the integrity of the corticospinal tract as measured by transcranial magnetic stimulation (TMS), and not to the spatial extent of the infarct. Further analysis of these data suggested that motor recovery (at 4 weeks) was best predicted by a covariance between the resting rCMRGlu in contralesional cerebellum and ipsilesional thalamus, a relationship not seen in patients with poor recovery (Azari *et al.*, 1996). Chollet *et al.*, (1991) also reported a functional covariation between the ipsilesional thalamus and contralesional cerebellum during an active motor task (as opposed to rest), when measuring rCBF using PET. Furthermore, Azari *et al.*, (1996) reported rCMRGlu interdependencies between ipsilesional thalamus, contralesional cerebellum and bilateral supplementary motor area in recovered patients, which were not seen in normal controls.

The diversity of results from the above studies may in part be accounted for by the use of different techniques on different groups of patients (cortical and subcortical infarcts) at different time points from stroke onset, and thus may be investigating different phenomenon. There is however some evidence to support the idea that improvement in diaschisis has functional significance and that areas of the brain remote from the site of damage may subserve recovery. In particular, it appears that a better outcome can be gained if certain deep structures, particularly thalamus, maintain their functional connections with cortical motor structures.

2.3 Motor task related activation studies

2.3.1 Early PET studies

The acquisition of functional imaging data on stroke patients whilst they are performing a motor task will provide information about the areas of the brain subserving movement. Patients have usually been studied after making a good recovery and any differences in activation patterns compared to controls have lead to inferences being made about the structures in the brain subserving this recovery.

The early functional imaging studies of motor recovery following stroke were performed at the Hammersmith Hospital, using PET. In the first, six patients who had recovered hand function were scanned whilst performing a finger opposition task, two months or more after a first ischaemic subcortical stroke. Compared to movement in the 'unaffected' fingers, movement of the recovered fingers was associated with bilateral activation in primary sensorimotor cortex. Bilateral

activation was also seen in premotor, inferior parietal, and insula cortex, as well as both cerebellar hemispheres (Chollet *et al.*, 1991).

In the second study, ten patients who had recovered hand function following first (striatocapsular) ischaemic stroke, three months or more previously were studied using the same motor paradigm, this time compared to ten normal subjects. Activation was seen in contralateral cortical motor areas and ipsilateral cerebellum as in normal subjects, but activation was greater than normals in prefrontal, inferior parietal, insula, and anterior cingulate cortex bilaterally, as well as ipsilateral premotor cortex, striatum and contralateral cerebellum (Weiller *et al.*, 1992).

The initial hypothesis formed was that sensorimotor cortex ipsilateral to the moving hand, as well as motor areas normally only recruited during complex motor tasks, were responsible for taking over the function of the damaged brain. In these two studies, results of several patients were analysed together. Methodological advances soon enabled individual patients to be studied and compared to a group of normal subjects (Weiller *et al.*, 1993). Eight patients, five with striatocapsular infarcts and three with anterior choroidal artery territory infarcts, who had recovered hand function following stroke between seven weeks and six years previously, were studied using the same finger opposition task. Not surprisingly in this heterogeneous group of patients (five had lesions involving the posterior limb of the internal capsule, one the genu and three the anterior limb), there was a certain amount of variation between subjects performing the same motor task. Compared to normal subjects, these patients activated combinations of ipsilateral premotor cortex and contralateral cerebellum, as well as bilateral

prefrontal, premotor, inferior parietal, insula, and anterior cingulate cortices and bilateral supplementary motor areas. In general there was greater activation of ipsilateral motor areas than in normal subjects, but ipsilateral primary somatosensory areas were activated in only four patients, each of whom displayed mirror movements, and so it was felt that this finding in particular could not be attributed to reorganisation of the motor system. The other notable feature of the results was that in patients with involvement of the posterior limb of the internal capsule, there was a ventral extension of the cortical representation of the hand into areas normally associated with face representation.

The later study by Seitz *et al.*, (1998) was notable for the fact that patients had infarcts involving the primary motor cortex, as compared to the subcortical stroke patients previously studied. Compared to controls, patients activated bilateral premotor cortex and supplementary motor area, but not primary motor cortex (Seitz *et* al., 1998).

Dettmers *et al.*, (1997) studied six stroke patients with incomplete recovery using a different paradigm. Rather than asking patients to perform simple finger tapping, they were asked to generate a range of forces, calibrated to the peak force of each patient's own maximum voluntary contraction, or MVC (5%, 10%, 20%, 40% and 46.7% of their own MVC). The authors described an altered relationship between force exerted in a finger press task and regional cerebral blood flow (rCBF) in patients when compared to normals. Ipsilesional sensorimotor cortex activity showed a binomial relationship with force compared to a logarithmic relationship in controls. Furthermore, linear increases in rCBF with increasing force, not seen in controls, were observed in SMA and parietal cortex. These observations

provide further evidence to support a functional role for alternative motor networks in patients regaining some motor function after stroke, as not only are these regions recruited, but they behave in a different fashion during a motor task.

2.3.2 Early fMRI studies

Similar experiments using fMRI have been conducted, and throw some light on the question of whether contralesional sensorimotor activations are related to mirror movements of the unaffected hand. Cramer *et al.*, (1997) studied ten patients who had suffered from cortical or subcortical stroke between eleven days and fifteen months previously, performing a finger tapping task during fMRI scanning. In normal subjects bilateral activations were seen in motor areas (more so on the contralateral side) and the same areas were activated by stroke patients when moving the recovered hand. However, a greater degree of activation compared to controls was seen in ipsilateral sensorimotor and premotor cortex, contralateral cerebellum, and bilateral supplementary motor area. Unlike the study by Weiller *et al.*, (1993) only one out of ten patients demonstrated mirror movements. Ipsilateral sensorimotor cortex activation was also seen in stroke patients by Cao *et al.*, (1998). Eight patients with partial recovery of hand function were studied between five and forty three months after (mainly) cortical strokes. A self paced finger opposition task was employed. Ipsilateral sensorimotor cortex was activated by movement of the paretic hand in six out of eight patients. This ipsilateral activation was not felt to be a consequence of mirror movements, as these were present in only two out of six patients with ipsilateral sensorimotor cortex activation, although this finding was based on observation

rather than electromyographic (EMG) recording. Furthermore, in one patient with mirror movements, there was exclusively ipsilateral activation during paretic hand movement, suggesting that the contralesional hemisphere was mediating movement in both left and right hands. This provides further evidence against contralesional activations being the result of mirror movements.

There are also data to support the notion that surviving peri-infarct cortical tissue may be helpful to the recovering patient. Cao *et al.*, (1994) studied teenage patients who had suffered a perinatal infarct, each with only moderate recovery. Sequential finger movements of the affected hand were associated with bilateral activations, as well as peri-infarct cortical rim activations. Peri-infarct cortical rim activations in recovered stroke patients were seen by Cramer *et al.*, (1997) during similar tasks.

In addition to peri-infarct activations, shifts in ipsilesional peak sensorimotor cortex activation have been observed in several studies. Cramer *et al.*, (2000) reported shifts in cortical maps in two patients with good recovery following mild cortical strokes involving either pre or post-central gyrus. Patients were studied during both simple motor tasks and tactile stimulation. I have already described the ventral shift in peak sensorimotor cortex activation observed by Weiller *et al.*, (1993), and subsequently an overall caudal shift has been reported in a group of recovered stroke patients by Pineiro *et al.*, (2001). Rossini *et al.*, (1998) also reported this shift of cortical hand representation using fMRI, magnetoencephalography and TMS, in a patient with recovered hand function following cortical stroke. There appears to be no consistent direction of this shift, and the observation has not been clearly linked to recovery. However, taking the

findings into consideration together with the data on perilesional activation, it appears that in some patients, recovery of motor function may be subserved by preserved cortex on the side of the infarct.

These studies have often been interpreted as demonstrating changes in functional anatomy that *underlie* recovery. However, until patients with little or no recovery have been studied, this cannot be said to be true. Other hypotheses for the variability of activation patterns following stroke should be considered. For example, Cao *et al.*, (1998) studied patients with incomplete recovery, and the patterns of activation seen did not seem to relate to degree of functional recovery, but seemed more closely linked to the site of the infarct. Three out of four cortical strokes led to contralesional sensorimotor activations with movement of the paretic hand. The two cases of subcortical stroke activated bilateral motor areas with movement of the paretic hand, and the patients with cortical lesions that spared the hand representation, activated predominantly ipsilesional sensorimotor cortex and contralesional premotor areas. Although the numbers are small, these data might suggest that the pattern of activation is simply a physiological consequence of the site of the lesion rather than evidence of a reorganised neural network underpinning motor recovery, as has been suggested by other investigators.

2.3.3 Early longitudinal studies

In order to determine whether changes in activation patterns are related to recovery, one might study the time course of these changes in relation to recovery. Marshall *et al.*, (2000) obtained longitudinal data, by performing fMRI scans

during a finger thumb opposition task on eight patients. The first scan was performed within one week of lacunar stroke, and the second at three to six months post stroke. In six patients there was complete paralysis at first assessment (such that they were unable to perform an active motor task), and by the second assessment all patients had fully recovered. It was therefore not possible to compare results over time with the degree of recovery. The pattern of activation with movement of the paretic hand (or attempted movement in the case of those with complete paralysis) altered from the first to the second scan. Specifically, the ratio of ipsilesional to contralesional sensorimotor cortex activations increased over time, i.e. returned to a more 'normal' lateralised pattern.

Calautti *et al.,* (2001a, 2001b) studied a similar case mix of five stroke patients at two time points using PET, although the first scan was performed somewhat later than in the study of Marshall *et al.*, (2000). These authors too, reported 'striking overactivations' at the first study, notably in the ipsilesional sensorimotor cortex (including caudal extension). At the second scan there appeared to be a relative decrease in activation of non-primary motor regions particularly in the affected hemisphere.

On the face of it, the results of these two studies (Calautti *et al.,* 2001b; Marshall *et al.*, 2000) are similar, both reporting decreases of activation in motor regions outside of contralateral (ipsilesional) motor cortex. However, both groups reported their results in terms of changes in the laterality index. The laterality index (LI) is a measure of the relative activation within one hemisphere compared to the other, or in a specified region (e.g. sensorimotor cortex) of one hemisphere compared to the homotopic region. It is calculated by counting the number of voxels which are

significantly activated at a given statistical threshold, and then LI = (contralateral – ipsilateral)/(contralateral + ipsilateral). A positive value indicates greater a number of activated voxels in the contralateral compared to the ipsilateral hemisphere, and negative value indicates the reverse. If one considers the changes in LI of the above two studies, there was a positive shift over time in one (Marshall *et al.*, 2000), and a negative shift in the other (Calautti *et al.*, 2001b). Examining the results in more detail, it appears that the reductions in activations in one study (Marshall *et al.*, 2000) were largely in contralesional hemisphere, and in the other study were largely in ipsilesional hemisphere (Calautti *et al.*, 2001b).

It is difficult to know how important the difference in results between these studies is. The first point to consider is that in the study by Marshall *et al.*, (2000), patients were performing different tasks at each time point, making interpretation of changes over time virtually impossible. Calautti *et al.*, (2001b) however were careful to ensure that the absolute parameters of the task remained the same, making direct comparison of the two time points perfectly valid. However, it could be argued that although the absolute task parameters remained the same, the *effort* required by a recovering patient would be less for the second scan session, and this would have to be considered when interpreting the results. The second point relates to the laterality index itself. The laterality indices in each study are not measuring the same thing. Marshall *et al.*, (2000) were interested in the change in laterality index for sensorimotor cortex, whereas Calautti *et al.*, (2001b) used whole hemisphere activations. This difference must affect the interpretation of the results. In addition the values obtained from this voxel counting approach are dependent on the threshold taken to represent statistical significance, which is

arbitrary. In addition, if activation in each whole hemisphere is compared, this takes into account many cortical regions, some of which may become increasingly involved, and others less involved during recovery. Lastly, it takes account of the extent of activated clusters, but not the peak size of activation. Use of the laterality index may provide some interesting biological information, but it would appear to be extremely difficult to interpret in a meaningful way. Having said that, Calautti *et al.*, (2001b) went on to demonstrate a striking positive correlation between changes in laterality index and changes in performance (as measured by thumb to index finger tapping rates). Although 4 out of 5 patients had a negative shift of the laterality index, those patients with greater improvement had less negative shift. It is difficult to know exactly what this result might indicate, but it was the first time that anyone had attempted to look for a link between task related activations and recovery, clearly an important approach.

Further longitudinal studies are required, but these studies demonstrate some of the difficulties involved. The approaches taken in the experiments in this thesis are discussed in the next chapter. An alternative approach to the problem of confounds arising due to changes in the ability to perform a task is to use a different task, such as passive movement of the paretic limb. This approach is discussed in the following section.

2.4 Passive movement studies

Experiments in which either the elbow or the wrist is passively flexed and extended in normal volunteers have tended to demonstrate activations in ipsilesional sensorimotor cortex, rostral supplementary motor area, and bilateral

inferior parietal cortex, when compared to rest (Weiller *et al.*, 1996; Carel *et al.*, 2000). This task may be performed at any stage of recovery, without performance confounds, and Nelles *et al.*, (1999) took advantage of this by studying passive movement of the elbow compared to rest in six patients with first pure motor subcortical stroke, using PET. Before hand or forearm recovery had occurred, passive movements lead to activations predominantly in the contralesional hemisphere (inferior parietal, dorsolateral prefrontal, cingulate, and premotor cortices). A second scan was carried out three weeks later when four of the patients had recovered control of forearm movements, but two had little recovery. Bilateral increases in rCBF were again seen in inferior parietal cortex, although now more so on the ipsilesional side, and strong activation was now apparent in ipsilesional sensorimotor cortex. Unfortunately single subject analysis could not be performed and so correlation with degree of recovery could not be made. Weiller *et al.*, (1997) attempted to study the predictive power of early PET scanning using a passive movement task. Two patterns were found by analysing the individual PET scans. In recovered patients, passive movement at the elbow compared to rest showed extensive activations similar to those demonstrated by Nelles *et al.*, (1999) whereas in those with poor recovery, activation was limited to motor cortex in the damaged hemisphere. Only eight patients were studied, while data on the location of the infarcts was not available. However it is nevertheless an interesting finding, suggesting that the preservation of proprioceptive input into cortical motor areas thought to be involved in recovery may play a role in restitution of the ability to perform willed movement. In animal models although there is an increase in the potential for plasticity in post lesioned brains, it is only

an increase in activity i.e. input into the system, that takes advantage of this and leads to an improvement in function (Schallert *et al.*, 2000). The studies of the central effects of passive movement provide an interesting link with this work.

2.5 Neurovascular coupling in cerebrovascular disease

In the normal brain, regional cerebral blood flow is tightly coupled to the local metabolic demand and therefore is sensitive to the regional neuronal activity. After stroke however, this relationship is disrupted (Ackerman *et* al. 1981; Lenzi *et al.*, 1982; Baron *et al.*, 1984), which is of theoretical concern as functional activation studies rely on the haemodynamic response to an increase in synaptic activity (see chapter 3). Wise *et al.*, (1983) attempted to define the time course of these changes, using PET after acute stroke. Nine patients were identified with elevated regional oxygen extraction fractions at the site of the lesion within the first 36 hours post stroke. These patients were scanned a second time, all within a week, and in eight out of nine, regional oxygen extraction fractions had fallen, either due to reperfusion or further impairment of mitochondrial function, or both. It would appear that these changes occur in at least 75% by 24 hours, and an elevated OEF with moderately reduced tissue oxygen metabolism is seen only rarely after 30 hours (Wise *et al.*, 1983).

The question of whether an uncoupling of flow and metabolism remains later than this, acting as a confound in any functional imaging experiment has been addressed using techniques to examine the relationship between vascular reserve and changes in rCBF due to neuronal activation. Inao *et al.*, (1998), studied six patients with symptomatic unilateral internal carotid artery or middle cerebral

artery steno-occlusive disease using PET. They were able to demonstrate increases in regional CBF in response to a simple motor task, in cortical areas in which no increase in regional CBF could be induced by the administration of acetazolamide. Reduced acetazolamide reactivity correlates well with elevated oxygen extraction fraction, and thus to reduced haemodynamic reserve (Hirano *et al.*, 1994). It would appear then that changes in regional cerebral blood flow induced by acetazolamide and those induced by neural activation are independent. Li *et al.*, (1999) have used an arterial spin-tagging technique based on flow sensitive alternating inversion recovery (FAIR), to similar effect, demonstrating that the regulatory mechanisms for the vasodilatory reaction to CO_2 and regional CBF response to neural activation using a motor task, are also independent. The BOLD signal of fMRI is however dependent not only on CBF but also on cerebral blood volume, metabolic rate of the tissue, and vascular configuration. Although it appears that in the normal brain the BOLD response to hypercapnia and to neural activation in the visual cortex are independent (Corfield *et al.*, 2001), this has yet to be demonstrated empirically early on after stroke. Functional imaging studies using BOLD fMRI techniques early after stroke will need to take this potential problem into account.

In summary, it appears that the disturbed relationship between cerebral blood flow and metabolism returns to normal within a few days after acute cerebral infarction. Furthermore neurovascular responses seem to be unaffected by disturbances in cerebral haemodynamic reserve. Thus, despite the absence of specific empirical data, it seems reasonable to proceed with functional imaging studies in post acute stroke patients within a week or two after the event. The

experiments in chapters 7 and 8 involved fMRI were performed no earlier than 10 days after infarction.

2.6 Conclusions

Functional imaging techniques provide an important tool with which to study the human brain at work. To date these studies do not provide a clear link between observed changes and functional recovery. Much more care needs to be taken over the collection of outcome data in the study of patients both with and without recovery. Ideally, longitudinal studies in individual patients should correlate the physiological data obtained with outcome measures. Furthermore, the design of these studies needs to carefully control for a number of factors, in particular performance confounds, and non-specific session effects. The experiments in this thesis were carried out with these issues in mind.

Chapter 3

METHODS AND MATERIALS

I. THE ACQUISITION AND ANALYSIS OF FUNCTIONAL IMAGING DATA

3.1 Introduction

A number of non-invasive techniques are now available for the study of the working human brain. This includes those that measure electrical (electroencephalography) or magnetic (magnetoencephalography) field changes at the scalp, and those that rely on a close relationship between neuronal metabolism and changes in cerebral blood flow at a local level, namely single photon emission computed tomography (SPECT), positron emission tomography (PET), and functional magnetic resonance imaging (fMRI). After addressing some general issues concerning the relationship between brain metabolism and cerebral blood flow, and the methodologies utilising this neurovascular response, the rest of the chapter will concentrate on the on the principles behind the acquisition and analysis of fMRI data sets.

3.2 The relationship between neuronal metabolism and cerebral blood flow

Functional imaging techniques rely on the measurement of changes in regional cerebral blood flow (rCBF) or flow-related phenomena, but how do these parameters relate to the underlying neuronal activity which is of interest to the neuroscientist? Under normal physiological conditions, the brain uses glucose as its only source of energy (Sokoloff, 1977; Fox *et al.*, 1988). As there is little in the way of glycogen stored in the brain, glucose must be delivered via the blood supply. At rest, approximately 85-90% of this glucose is used by neurons to produce adenosine triphosphate (ATP), rather than by the supporting glial cells, and the proportion may increase during 'activation' (Clarke and Sokoloff, 1994). Furthermore, several groups have confirmed that it is the presynaptic terminal that is the principal site of enhanced metabolic demand (Kadekaro *et al.*, 1985, 1987; Nudo and Masterton, 1986). Crucially, local cerebral glucose metabolism is tightly coupled with local oxygen metabolism and local cerebral blood flow (for review see Jueptner and Weiller, 1995), although the exact mechanism whereby the cerebral blood flow changes locally is still debated. The result however is that the measurement of changes in regional cerebral blood flow (rCBF) or flow-related phenomena reflects (presynaptic) neural activity.

3.3 Quantitative rCBF measurement

Kety and Schmidt (1945) initiated the study of human cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$). They developed a technique requiring the introduction of an inert, diffusible and soluble tracer such as nitrous oxide into the arterial system, together with simultaneous arterial and venous sampling, which

enabled them to measure both CBF, the cerebral arterio-venous oxygen difference (A-V O_2) and arterial oxygen concentration and thus $CMRO_2$. They demonstrated the capacity of the normal brain to extract appropriate quantities of oxygen for metabolic need over a wide range of blood flows produced by hypercapnia (Kety and Schmidt, 1948), suggesting that for any $CMRO_2$ there is an appropriate, coupled CBF. An inappropriate fall in blood flow is accompanied by increased oxygen extraction and an increase in A-V O_2 to maximal values, the hallmark of ischaemia. Sokoloff (1966) studied a group of asymptomatic elderly subjects described as having arteriosclerosis, and found a decline in CBF compared to normals, with an elevated A-V O_2 suggesting a haemodynamically compromised state. Inferences from such early studies were hampered by the fact that measurements represented only global cerebral blood flow.

Methods for measuring mean hemispheric cerebral blood flow or regional cerebral blood flow (rCBF) were soon introduced and depended on the external detection of inert, freely diffusible, radioactive tracers such as xenon-133 (^{133}Xe). Early studies relied on bolus injection into the internal carotid artery, but Mallett and Veall (1963) described the first non-invasive regional technique using ^{133}Xe inhalation followed by clearance detection. The accuracy of techniques using ^{133}Xe was relatively poor. This was because of contamination of the signal by activity in the extracerebral tissues and air passages when the inhalation technique was used, and because recirculation of ^{133}Xe distorted the clearance curves. Further refinements were made to these techniques including the use of intravenous tracers. The poor imaging characteristics of ^{133}Xe lead to alternative tracers being used. N-isopropyl-p-^{123}I iodoamphetamine (^{123}I-IMP) was found to

have a distribution reflecting regional CBF (Kuhl *et al.* 1982), and subsequently technetium-99m hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO) has been extensively used. These two techniques can provide images of blood flow distribution, but are not able to provide accurate absolute quantification of CBF.

Positron emission tomography (PET) is a tool that enables regional brain function to be assayed in a fully quantitative manner. Biological probes are radioactively tagged with positron emitting isotopes, which are produced by a cyclotron. These labelled compounds are introduced into the body, usually intravenously, after which the positrons rapidly lose energy in collisions with electrons in the tissue. The masses of the positron and of the electron are converted into energy in the form of two gamma rays, which are emitted in opposite directions. The PET scanner consists of circumferential arrays of detectors designed to look for such a simultaneous emission of gamma rays in opposite directions. Depending on the isotope selected and the method of introduction into the body, the measurement of the concentration of positron labelled molecules in the brain can be used to infer regional cerebral blood flow, glucose metabolism, or even receptor distribution (Cherry and Phelps, 1996). PET and SPECT will not be discussed further, as neither was used to acquire data in this thesis.

3.4 Functional magnetic resonance imaging

Functional magnetic resonance imaging (fMRI) is a special form of magnetic resonance imaging (MRI), which relies on the principle of nuclear magnetic resonance (NMR), established over fifty years ago (Purcell *et al.*, 1945; Bloch *et*

al., 1946). Its use in medical imaging started only in the 1970's, and whole body MRI scanners started to be used in hospitals in the early 1980's. The technique of fMRI was soon developed, and has been used increasingly ever since in the field of systems level neuroscience. Some of the basic principles of MRI are now described.

3.4.1 Proton spin

Hydrogen constitutes approximately one tenth of the average human body mass. Nearly three quarters of this is contained in water molecules, and nearly one quarter in fat. The nucleus of a hydrogen atom is a single proton. This proton is not stationary but possess a property known as *spin*. Spin may be positive or negative, and charged particles such as protons and electrons may combine in such a way that cancels out the net effects of each. However, nuclei with an odd number of particles such as the hydrogen nucleus (the proton) will generate a net magnetic field, **M,** along the spin axis. In the presence of an externally applied magnetic field, **B**, such particles will align themselves with the direction of the field, but their spin properties prevent this alignment from being perfect. The spin axis itself will rotate or *precess* around the direction of the magnetic field (figure 2.1), in the same way that a spinning top will precess around the earth's gravitational field. The frequency of precession is proportional to the strength of the magnetic field, and is also related to the type of nuclei within the field. These parameters are related by the *Larmor equation*:

$$\omega_0 = \gamma B_0$$

where ω_0 is the precession frequency (radians per second), $\mathbf{B_0}$ is the flux density of the external magnetic field (Tesla, T), and γ is a gyromagnetic constant for each type of nucleus. The precession frequency in Hz, or the Larmor frequency, f is described in the following equation,

$$\omega_0 = 2\pi.f$$

and so,

$$f = \gamma B_0/2\pi$$

and for hydrogen protons,

$$f = 42.57\ B_0\ \text{MHz}$$

When placed in an external magnetic field, precessing protons will align themselves parallel to, or antiparallel to this magnetic field. The former is a low energy state, and the latter a high energy state. The population magnetic vector (sum of all spins) for a group of protons points in the direction of the applied field (longitudinal magnetisation) as there is a slight excess of protons in the low energy state. Each individual proton also has a component of magnetisation that is at right angles to the external applied magnetic field because of its own precession. Because the precession of each proton is in a different phase however, there is no net transverse magnetisation. Thus at equilibrium the net magnetisation vector $\mathbf{M_0}$ lies in the direction of the applied external magnetic field $\mathbf{B_0}$.

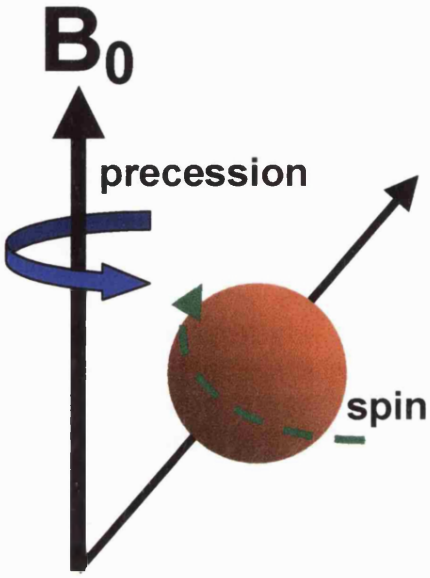


Figure 3.1 The spin and precession of a single proton. Direction of spin indicated by green arrow. Precession around longitudinal magnetisation vector (B_0) of applied external magnetic field, indicated by blue arrow.

3.4.2 Generating MRI signal

MRI relies on perturbing the magnetic equilibrium of precessing protons and measuring the resulting signal. This can be achieved by applying radiofrequency (RF) pulses to generate a weak magnetic field $\mathbf{B_1}$, usually perpendicular to $\mathbf{B_0}$. The RF pulses are delivered at a particular frequency, which transfers energy to the precessing protons. The principle of resonance dictates that in order for the transfer of energy to be optimised the RF frequency should be equal to the Larmor frequency. This process results in the shift of some protons to the higher energy state, often termed *flipping*. Consequently the net longitudinal magnetisation vector is reduced. This cannot be directly measured, as it is in the same plane as the applied magnetic field, $\mathbf{B_0}$. In addition, the phase of the precessing protons becomes synchronised, resulting in the generation of a net transverse

magnetisation vector. This in-phase precession is crucial, as it is the origin of the MR signal. Delivery of the RF pulse is referred to as the *excitation* phase.

The MR signal will be maximal immediately after excitation. However, if the MR signal is measured some time after the RF pulse is turned off, then there will be some decay in the signal as protons tend to *relax* back to their equilibrium states. The longitudinal and transverse magnetisation vectors will therefore return to their original values. The longitudinal magnetisation vector will increase back to its original value, a process termed T1 relaxation, or *spin-lattice relaxation*, in reference to the transfer of energy from the proton to surrounding local tissue. Spin-lattice relaxation is governed by the T1 time constant, where T1 is defined as the time to reduce the difference between longitudinal magnetisation vector ($\mathbf{M_L}$) and its steady state value ($\mathbf{M_0}$) by a factor of e. This process can be described as:

$$\mathbf{M_L = M_0(1-e^{-t/T1})}$$

The composition of the surrounding tissue will of course affect T1. So for example, protons in fat can transfer energy quicker than those in water, as the carbon-carbon bonds in fat resonate at a frequency much closer to that of the Larmor frequency.

During relaxation, the transverse magnetisation vector will also alter, decreasing back to its original value (i.e. zero) as precessing protons become less synchronised. This process is termed T2 relaxation, or *spin-spin relaxation*, in reference to the transfer of energy from the proton to surrounding protons. Spin-spin relaxation is governed by the T2 time constant, where T2 is defined as the

time to reduce the difference between transverse magnetisation vector ($\mathbf{M_T}$) and its steady state value ($\mathbf{M_0}$) by a factor of e. This process can be described as:

$$\mathbf{M_T = M_0 e^{-t/T2}}$$

In a local magnetic field that is not homogenous, the Larmor frequencies of protons will vary, and so will lose coherence (thus reducing $\mathbf{M_T}$) more rapidly than predicted. The relationship between the T2 time constant, and the effective T2 time constant, or T2*, is given by,

$$\mathbf{1/T2^* = 1/T2\ (molecular\ effects) + 1/T2\ (B_0\ field\ effects)}$$

In summary, when the RF excitation pulse is switched off, the combination of T1 and T2 effects results in a return of the net magnetisation vector to the equilibrium position, and this is described by a spiralling motion around the $\mathbf{B_0}$ axis. The precessing transverse magnetisation vector constitutes a changing magnetic field, and will therefore induce an electromagnetic field in a detector coil, which forms the MR signal. The frequency of this signal is equal to that of the applied RF pulse, which is equal to the Larmor frequency, and is in the radiofrequency bandwidth of the electromagnetic spectrum. T1 and T2 relaxation times are fixed for a given nucleus in a given environment. The time available for relaxation can be varied by altering the scan repeat time (TR) and the time between delivering the RF pulse and measuring the resulting signal (TE). The consequent change in

signal strength, which is dependent on the relaxation times, will provide contrast between different tissue types. A short TR and TE will emphasise T1 characteristics, whereas a long TR and long TE will emphasise the T2 characteristics.

3.4.3 Image formation

This section describes how the signal generated by MRI can be manipulated in order to produce three dimensional images of whole brain. Placing a sample within an homogenous magnetic field ($\mathbf{B_0}$) will result in the same magnetic field being experienced by all protons in that sample. As such, the frequency of the emitted signal resulting from a RF pulse ($\mathbf{B_1}$) will be the same for all protons. In MRI therefore, a second magnetic field (a gradient field) is applied along $\mathbf{B_0}$, which introduces a gradient along this axis. By applying the Larmor equation, it can be seen that protons will precess at different frequencies according to their position along this gradient. Thus the distribution of spin frequencies will vary along $\mathbf{B_0}$ axis depending on the spin density. Combining this gradient field with an excitatory RF pulse of narrow frequency bandwidth, will excite, or resonate with, a narrow slice of the sample, i.e. those protons precessing with the same frequency as the RF pulse. The gradient field may be applied along any axis (termed the *z*-axis) in order to define a plane or slice of interest. Acquisition of signal immediately after the slice selection pulse would result in a one-dimensional projection of spin densities in the selected slice, but to generate two-dimensional information which allows localisation within the slice, gradient fields are also generated in the *x*- and *y*-axis.

The effect of a second gradient in the *x*-axis (from one side of the head to the other) is to cause protons at different locations to precess at different frequencies. This is termed the *frequency encoding gradient.* The gradient in the *y*-axis (from front to back of the head) has the effect of imparting a phase difference in the spins of protons which relates to their position in the gradient field. This is termed the *phase encoding gradient.* Thus the position of a proton in each slice may be determined by a combination of frequency information read along the *x*-axis, and phase information along the *y*-axis. This information is transformed from the time domain to the frequency domain, by means of a Fourier transformation. MRI data for each slice may be considered as lying in two dimensional Fourier space. This is referred to as *k* space, and data within *k* space corresponds to MR data before transformation into an image.

Functional MRI requires ultra-fast image acquisition. Typical MRI sequences, such as spin-echo sequences can take minutes to acquire each slice, but the introduction of echo-planar imaging (EPI) (Mansfield, 1977) sequences reduced this time to fractions of a second. The major difference between EPI and other MR acquisition sequences lies in the way that *k* space is sampled. A typical MR sequence will sample one line of two dimensional *k* space after each RF pulse, whereas EPI measures all lines of *k* space simultaneously after a single excitation. EPI is therefore the sequence of choice for fMRI experiments that require the accurate measurement of rapidly changing signal, and has been used in all experiments described in this thesis.

3.4.4 Blood oxygen level dependent (BOLD) signal in fMRI

As discussed above, the effective T2 time constant, or T2* is influenced by interactions between precessing protons themselves and also by magnetic field inhomogeneities induced by the different magnetic properties of surrounding molecules. The magnetic properties of some molecules may change depending on the state of the molecule. For example, the magnetic properties of haemoglobin, the protein responsible for carrying oxygen within red blood cells, depend on its level of oxygenation. When not carrying oxygen (deoxyhaemoglobin) four electrons remain unpaired, thus generating a net magnetic moment, which is not present when oxygen is bound (oxyhaemoglobin). Deoxyhaemoglobin is therefore said to be paramagnetic. The net magnetic moment creates a local magnetic field gradient, which contributes to the decay of transverse magnetisation, thus shortening the T2* decay time. It can therefore be seen that changes in the ratio of oxyhaemoglobin to deoxyhaemoglobin will result in local changes in T2*. Experimental evidence to support this theoretical assertion was provided by Ogawa and colleagues (Ogawa and Lee, 1990; Ogawa *et al.*, 1990), and by Turner and colleagues (Turner *et al.*, 1991). These studies demonstrated that manipulation of the ratio of oxyhaemoglobin and deoxyhaemoglobin in animal models, led to detectable changes in signal in blood vessels and within the water tissue surrounding these vessels. Subsequently, similar evidence was obtained *in vivo* in human studies and termed blood oxygenation level dependent (BOLD) contrast (Kwong *et al.*, 1992; Ogawa *et al.*, 1992).

The BOLD signal is a consequence of the haemodynamic response to changes in neuronal activation. The exact relationship between the two is still debated, but

work using optical imaging techniques to study this neurovascular coupling suggests three distinct phases after an increase in neuronal firing. Firstly, the increase in neuronal firing in focal regions of cerebral grey matter leads to an increase in local oxygen uptake and a local decrease in blood oxygenation after approximately 100ms (Vanzetta and Grinvald, 1999). The local deoxygenation leads to vasodilatation and increased blood flow in the capillary bed around 300-500ms later. Consequently, the delivery of oxygenated haemoglobin to active neurons exceeds metabolic demand at about 500-1000ms after neuronal activity starts and so the ratio of oxyhaemoglobin and deoxyhaemoglobin alters, with a relative reduction in the paramagnetic deoxyhaemoglobin in the local capillary bed, and draining venules. The initial increase in deoxyhaemoglobin (leading to an initial dip in BOLD signal) is well localised to the site of neuronal activity, but the later decrease in deoxyhaemoglobin (and increase in BOLD signal) is spread over a less well defined area. Theoretically the initial dip in BOLD signal would provide better localisation of signal, but it has not been reliably detected at field strengths less than 4 Tesla (Hu *et al.*, 1997). The later decrease in deoxyhaemoglobin is approximately four times greater than the initial increase, and so is the major factor in the production of the BOLD signal.

3.4.5 Interpretation of the BOLD signal

The increased metabolic demand associated with the increased firing of a single neuron occurs whether the neuron is excitatory or inhibitory (Nudo and Masterton, 1986). Most of this energy consumption is at the level of the presynaptic terminal. However, fMRI experiments measure the BOLD signal in voxels that contain millions of neurons. The BOLD signal in each voxel will therefore reflect the net neuronal activity in that voxel, whether excitatory or inhibitory. However, although activity in inhibitory neurons or interneurons increases metabolic demand, the down stream effect will be of a reduction in synaptic activity, thus reducing the net metabolic demand, and therefore BOLD signal in that voxel. Thus BOLD signal reflects largely excitatory effects.

The observation that neurally evoked decreases in deoxyhaemoglobin are less spatially focussed than the initial increase raises a question about the relationship between the BOLD signal and underlying neural activity that it reflects. This question was addressed empirically by Logothetis and colleagues performing simultaneous measurements of BOLD, neuronal spike rates and local field potentials during visual stimulation in anaesthetised macaque monkeys (Logothetis *et al.*, 2001). Their results demonstrated that the BOLD signal correlates with local field potentials more closely than with spike rates, local field potentials reflecting sub-threshold integrative processes as well as local spiking. This result suggests that BOLD signal measured in fMRI experiments reflects afferent input and local processing within a cortical region during a particular task, rather than efferent signal as represented by neuronal spiking activity. This is an important result, as it is suggests a distinction between the underlying

physiological processes leading to the signal in an fMRI experiment and the signal obtained from single cell recording studies, which are often used to generate anatomical hypotheses for fMRI studies. In other words, spiking activity is reflected in the BOLD signal, but cannot fully predict it.

Thus, the BOLD signal originates largely from the capillaries and venules draining the capillary beds in grey matter. As this represents only a small fraction of the total grey matter, it is clear that BOLD signal changes are in the order of a few percent only. In addition, the measured neurovascular response occurs a number of seconds after the preceding neuronal event. So although fMRI images can be acquired rapidly in fractions of seconds, the effective temporal resolution of the data is determined by the smoothing inherent in the neurovascular response. This temporal smoothing must be taken into account when attempting to model the data for the purposes of statistical analysis and inference.

The raw data derived from fMRI may be contaminated by several sources, and in order to be able to confidently make inferences about true task induced signal change, rather than noise, the data need to be processed, before being subjected to statistical analysis. This process is described in the next section.

3.5 Spatial pre-processing

A functional magnetic resonance experiment will generate a number of three dimensional brain volumes, each made up of a number of volume elements or *voxels*, each with an intensity value that changes across the course of a single scanning session. In the case of fMRI this intensity value represents the average

BOLD signal over the volume of brain that the voxel covers. Thus, fMRI experiments generate a time series of BOLD signal changes for each voxel, and these time series provide the substrate for analysis. However, before this analysis can be carried out, a number of critical pre-processing steps must be carried out. All imaging data acquired during the experiments presented in this thesis, underwent spatial pre-processing as implemented in Statistical Parametric Mapping software (SPM99, Wellcome Department of Imaging Neuroscience, http://www.fil.ion.ucl.ac.uk/spm/) (Friston *et al.,* 1995a; Worsley and Friston, 1995) utilising Matlab5 (The Mathworks Inc., USA) as described in detail below.

3.5.1 Spatial realignment

The first step is one of spatial realignment. For a time series of BOLD signal change to represent true change in signal in a given brain region or voxel, it is necessary that the position of each voxel with respect to the scanner remains unchanged throughout an experiment. Since this is unlikely to be the case, it is necessary to reduce the effects of head movement as much as possible using an algorithm that realigns all images to the first image of each session. This potentially complex problem is achieved by treating each image as a series of points in three dimensions, thus rendering the problem solvable by linear algebraic methods. The differences between images are described by six parameters: three translations and three rotations about orthogonal axes. Images then undergo iterative rigid body transformations (such that the spatial relationship between points or voxels within an image remain constant) defined by these six parameters

until the sum of squares difference between the two images is minimised (Friston *et al.*, 1995b).

3.5.2 Unwarping

The realignment step is however not perfect. In particular, time series that involve a significant motor response are often left with some variance that is largely attributable to the task related movement. Traditionally, experimenters have included the six realignment parameters created during the steps described above in the design matrix (see section on design matrices below) in order to account for this variance. However if the motor task is the experimental manipulation of interest, then it is possible that true experimentally induced variance will be lost or at least reduced. The studies described in this thesis all employ isometric dynamic hand grip, and so although no subject moved more than 3 mm in any direction (and rarely more than 2 mm), some of this movement was task related. In order to remove some of this unwanted movement related variance without removing variance attributable to the motor task an alternative strategy has been described by Andersson and colleagues (Andersson *et al.*, 2001) and implemented in SPM99. Thus realigned images are processed using the 'unwarp' toolbox in SPM99 that is predicated on the assumption that susceptibility-by-movement interactions describe more accurately a sizeable part of residual movement related variance. Given the observed variance (after realignment) and the realignment parameters, estimates of how deformations changed with subject movement were made. These were subsequently used to minimise movement related variance.

Realigned, unwarped images were then resliced with sinc interpolation between neighbouring voxels.

3.5.3 Slice timing

The acquisition of data representing a whole brain volume takes several seconds, with slices being acquired sequentially from the top of the head down. Analysis of BOLD signal time series assumes that all data was acquired at the same time point. It can be seen that this is not the case, since within one volume data at the top of the head are acquired earlier than data at the bottom. To correct for their different acquisition times, the signal measured in each slice is shifted relative to the acquisition of the middle slice using sinc interpolation in time.

3.5.4 Normalisation

During the realignment stage, a mean functional image is created. This mean image is used to estimate the parameters necessary to warp each of the functional images into a standard EPI template based on the Montreal Neurological Institute (MNI) reference brain in Talairach space (Talairach and Tournoux, 1998). Warping is essentially a spatial transformation that acts to change the global shape of the subject's brain while preserving the relationships of its structures. At present, the estimation is achieved using a 12 parameter affine transformation where the parameters constitute a spatial transformation matrix (the affine transformation is similar to that used during realignment but includes zooms and shears). This estimation is conducted in a simple Bayesian framework, in which

an estimation is made of the deformation parameters θ that have the maximum posterior probability $p(\theta | y)$ given the data y, where

$$\mathbf{p(\theta | y) = p(y|\theta)\ p(\theta)}$$

The deformation is updated iteratively to maximise $p(\theta | y)$, which involves jointly minimising the likelihood and prior potentials. The likelihood potential is the sum of squared differences between the template and the deformed image that reflects the probability of actually getting that image if the transformation were correct. Prior information about the likelihood of a given transformation is incorporated by weighting the least squares (Ashburner *et al.*, 1997). The process of using the likelihood potential can be extended, and taken as differences between the index image and the best linear combination of templates depicting grey matter, white matter, cerebrospinal fluid and skull partitions. This approach allows differences in intensity that are not attributable to registration differences to be modelled. Furthermore, it provides the basis for allowing co-registration between different imaging modalities, e.g. T2* and T1 weighted images (i.e. functional and structural images for the purposes of examining the precise anatomical localisation of a statistical change in BOLD signal, in a single subject).

Thus realigned images are normalised and resampled to 3 x 3 x 3 mm^3 voxels. This process may result in incorrect normalisation (and therefore incorrect localisation of activations) in brains with abnormal structure, such as those with cerebral infarction or cortical malformation. In order to take account of this in our

stroke patients, a lesion mask was created (MRIcro, Nottingham University, http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html). This mask was then incorporated into the normalisation step for all patients (Brett *et al.*, 2001), which simply excludes the abnormal brain structure from the estimation process.

3.5.5 Smoothing

All normalised images were then smoothed with an isotropic 8 mm full-width half-maximum Gaussian kernel. Smoothing is performed to (i) allow valid statistical inference according to Gaussian random field theory (Friston *et al.*, 1995b), (ii) improve signal to noise ratio by smoothing using a kernel which corresponds to the expected size of the anticipated effect (matched filter theorem), and (iii) to help take account of inter-subject differences in functional anatomical localisation.

3.6 Characterising the haemodynamic response using the General Linear Model

The pre-processed images are now ready for statistical analysis. This proceeds on a voxel by voxel basis, using univariate statistical parametric tests to generate t- or F- statistics. These voxel by voxel statistics are then assembled into a 3-dimensional image that may be interpreted as a spatially extended statistical process. The second step is to draw inferences from these statistics in a manner that addresses specific prior hypotheses and reliably protects against false positive results. The following is an account of how these steps are implemented in

Statistical Parametric Mapping software (SPM99, Wellcome Department of Imaging Neuroscience, http://www.fil.ion.ucl.ac.uk/spm/).

3.6.1 Parameter estimation using the General Linear Model

The common parametric tests, including linear regression, correlation, t-tests, and analysis of variance (ANOVAs), are all single cases of a unified approach termed the general linear model (GLM). The GLM attempts to explain the data Y, as a linear combination of independent explanatory variables, plus an error term. Considering the case for the fMRI data generated at each voxel, the data consists of the changing BOLD signal, Y_j, where j is the number of observations of Y, and can be denoted,

$$\mathbf{Y}_j = \mathbf{x}_{j1}\boldsymbol{\beta}_1 + \ldots + \mathbf{x}_{jl}\boldsymbol{\beta}_l + \ldots + \mathbf{x}_{jL}\boldsymbol{\beta}_L + \boldsymbol{\varepsilon}_j$$

where L is the total number of explanatory variables, x_j. The explanatory variables are often the experimentally induced effects, but may also refer to effects of no experimental interest, but which may have contributed to changes in the observed data, Y. The explanatory variables are referred to as *covariates*. The errors, ε_j, are assumed to be independent and normally distributed, with zero mean and variance σ^2. Although σ^2 may vary across voxels, it is assumed to be constant across subjects. Thus to fit the model consisting of explanatory variables, x_j, to the data, Y, the parameters of the model are estimated. These parameter estimates are denoted β_L representing the unknown parameter for each of L explanatory variables. For the case where L=1,

$$\mathbf{Y}_j = \mathbf{x}_{j1}\mu + \mathbf{x}_{j2}\beta_2 + \varepsilon_j$$

such that $x_{j1}\mu$ is the intercept, and β represents the regression slope. However, when both *L* and *j* are greater than 1, the GLM can be expressed

$$\mathbf{Y} = \mathbf{X}\beta + \varepsilon$$

Where Y is a column vector (of size *j*) of observations, β a column vector (of size *L*) representing the weighting of each explanatory variable, i.e. parameter estimates, and ε a column vector (of size *j*) of error terms. X is a matrix of size *j* by *L*, and is termed the *design matrix*. The experimenter specifies the design matrix in order to account for as much of the variance in Y as possible. Consequently, the error term will be minimised, and statistical power improved.

The next stage is to estimate the parameters of the model. The optimal values of the parameters are those that minimise the total distance between the model and the data, which is calculated as the sum of the squared differences between each observed measurement (the data) and its estimate. The estimated (denoted by the addition of the ^ symbol) parameters that minimise the sum of squared errors are given by $\hat{\beta}$ where,

$$\hat{\beta} = (\mathbf{X}^T\mathbf{X})^{-1}\mathbf{X}^T\mathbf{Y}$$

This equation is only soluble if X^TX is invertible, i.e. there is a unique solution to $(X^TX)^{-1}$. This is only possible if none of the columns in the design matrix, X, can be formed by a linear combination of any of the other columns. In this case X is

said to be of full rank and if not, it is over-determined. For the over-determined matrix, X, there are an infinite number of matrices whose inverse is X, and so an infinite number of parameter estimates. If X is re-parameterised such that all the explanatory variables are mean corrected, i.e. sum to zero, then it is possible to use instead the pseudoinverse of X to constrain the infinite set of parameter estimates. This is the approach used in SPM.

After fitting the model, the residual variance σ^2 is estimated by the residual mean square as calculated by the residual sum of squares divided by the degrees of freedom (given by J-p, where p is the rank of X). As already stated, the errors are assumed to be independent and normally distributed across explanatory variables, with zero mean and variance σ^2.

3.6.2 The design matrix

It is clear from the above discussion that the key steps in ensuring minimum residual variance and therefore maximal statistical significance are careful experimental design and the subsequent construction of the design matrix, X. It is important to attempt to account for all variations in the measures BOLD signal, whether experimentally induced or not. Each independent explanatory variable is represented by a column in the design matrix.

3.6.2.1 Experimentally induced variables

A standard approach in experimental design would be to 'block' the task of interest into a defined time period (typically 20 seconds) alternating with a baseline. Thus the BOLD signal may change between the active block or epoch,

and the baseline epoch. This may be modelled with a boxcar function, consisting of ones for the active task, and zeros for the baseline. The use of a boxcar function requires that the blocks are of equal length, and that if discrete events occur within these blocks, that there should be an equal number of them within each block.

Alternatively the event of interest may not occur in blocks, but sporadically throughout the experiment. In such a case, each event is modelled not as a boxcar, but as a delta function or stick function of very brief length (effectively temporal singularity, and height one).

All the motor experiments in this thesis used a combination of the epoch and event-related designs. The motor task (isolated dynamic hand grip) was performed in epochs of approximately 20 seconds, followed by 20 seconds rest. Hand grips were modelled as events within blocks. The advantage of such an approach is that it allows the parameterisation of each event according to some other criteria. In the case of my experiments, each delta function was also parameterised according to the peak force exerted during that hand grip. These parameters form separate covariates. Such an approach allows a more comprehensive and realistic model to be built, such that the residual error is truly dominated by noise rather than unmodelled, experimentally induced variance. Any unmodelled, experimentally induced variance will contain some structure and thus contradict an assumption of the GLM.

The covariates described above are expected to produce a change in the BOLD signal. As previously described, there will be a lag between changes in neural activity and changes in the neurovascular response. Thus boxcar functions, delta functions, and their parameterised covariates, are convolved with a canonical

haemodynamic response function (HRF). This HRF is defined by the sum of two gamma functions with empirically determined onset, peak and width (Friston *et al.*, 1998). The resulting function is entered into the design matrix.

3.6.2.2 Non-experimentally induced variables

It may be possible to account for differences in signal because of effects other than those induced by experimental manipulation. In the case of multiple subject design matrices it is important to account for these between subject differences by modelling them. Realignment parameters are often included as covariates of no interest to account for differences in head position from one scan to the next. More recently unwarping algorithms have been used (and are employed in all the experiments in this thesis as described above) in the pre-processing stage for this purpose (see Section 2.5.2).

Although modelling all of these effects will help to reduce the residual variance, as well as normalise its structure, the inclusion of extra columns in the design matrix will also reduce the degrees of freedom. In an fMRI experiment with between 200 and 600 brain volumes, this is usually not a significant problem. However, a process known as model selection can determine the optimal model. Forward model selection refers to the process of starting with the simplest model, and adding covariates, or columns, to the design matrix one by one, and examining the effect using an F-test. Backward model selection proceeds from a complex model in the opposite direction, removing columns one by one. The process of forward model selection was employed in the experiment described in chapter 5.

3.7 Modelling fMRI time series

An fMRI experiment produces a BOLD time series in each voxel that contains additional effects that must be modelled. Firstly, the MRI scanner itself introduces noise into the experimental data. Secondly, the temporal dynamics of the HRF must be considered.

FMRI data is collected as a continuous time series, with experimental conditions occurring at a certain frequency (determined by experimental design). It is important to know this frequency as the distribution of noise (from which we wish to distinguish our experimentally induced changing signal) across all frequencies contains some structure, or correlations. It was proposed that the noise spectra conformed to a $1/f$ (frequency) model, suggesting that the power of the noise is greatest at lower frequencies (Zarahn *et al.*, 1997). Low frequency noise components are largely attributable to scanner drift or periodic physiological effects (Turner *et* al., 1993), but also possibly to learning effects. As a result, assumptions about error structure are violated, and fMRI designs where the experimental effect is of a similar frequency to the noise, will clearly be inefficient.

There is an additional issue that requires attention in the modelling of time series. The underlying transient neural activity has been temporally smoothed by the delayed neurovascular response, so that signal in one scan will be contaminated by signal from previous scans, i.e. it is not independent. This is described as *temporal autocorrelation.* To a certain extent this problem can be dealt with by careful experimental design. An approach that is embedded in SPM is temporal filtering, or smoothing. By smoothing with a filter that approximates the

frequency spectrum of the evoked haemodynamic response, there will be little effect on the experimentally induced variance and efficient attenuation of noise. The version of SPM employed in the experiments in this thesis (SPM99) employs bandpass filtering to achieve temporal smoothing, using a convolution matrix K that effects both low- and high-pass filtering simultaneously. The smoothing kernel K is applied such that,

$$\mathbf{KY = KX\beta + K\varepsilon}$$

By choosing a kernel of larger size than the endogenous autocorrelation, it is assumed that autocorrelations will be swamped by K (Friston *et al.*, 1995c). Because the data are not independent the effective degrees of freedom are lower than J-p, and in order to make correct statistical inferences, the effective degrees of freedom are recalculated from the known autocorrelation structure in the collected data.

3.8 The process of statistical inference

3.8.1 Statistical parametric maps

The eventual result derived at each voxel from the above steps is a t- or F-statistic. Experimental questions usually demand that a subspace of the design matrix be explored in order to address specific null hypotheses. This is achieved by using a vector of contrast weights, which weight the parameter estimates of the covariates of interest. In the case of the null hypothesis that there is no greater brain

activation during task A (modelled by covariate A) than in task B (modelled by covariate B), the vector of contrast weights will weight the parameter estimate for covariate A by +1, and the parameter estimate for covariate B by −1. All other covariates will receive 0 weighting. The chance of a there being significant difference in brain activation in these two conditions is given by a t-statistic that is dependent not only on the magnitude of the parameter estimates, but also the error variance at that voxel across the whole set of J scans. The t-tests used in SPM are one tailed. The second tail represents the reciprocal contrast (B vs A) that is normally tested for separately as another hypothesis. The F-statistic can answer a different sort of question. It tests the null hypothesis that a covariate or combination of covariates does not explain any of the observed variance. Thus the weighted combination of parameter estimates is divided by the error variance of the whole model. The F-test is two-tailed, and is best used during forward or backward model selection, or when no specific hypothesis about the direction of an effect or difference exists. Having obtained a t- or F-statistic at each voxel, these values can be colour coded and displayed on 3-dimensional brain volumes (usually T1-weighted images) to produce statistical parametric maps, or SPMs.

3.8.2 Experimental models

The design matrix can be constructed in a number of ways depending on the experimental question. Similarly, the contrasts used may vary depending on the subspace of the design matrix that is of interest. The simplest example would be to subtract the rest scans from the scans during an active task. Where there is more than one active task, the difference between two of these tasks might be

'characterised by separable cognitive or physiological components and the locations of rCBF (or BOLD) differences associated with the two tasks associated with areas functionally specialised for these components' (Frackowiak and Friston, 1994). This process is termed *categorical comparison,* and is still much in use. The results are only meaningful if the experiment has been designed in such a way that the two states differ only by a cognitive or other component of interest, and makes the assumption that this component may be purely inserted or withdrawn with no resulting interaction. This approach has been used to examine the main effect of hand grip compared to rest in the experiments described in chapters 5, 6, 7, and 8.

A second approach to examining the design subspace is that of *parametric analysis* which adopts a correlational approach. The central tenet of this approach is that regional signal (whether rCBF of BOLD) will vary monotonically and systematically in parallel with changes in a specific task related parameter, such as peak force of hand grip or frequency of aural word presentation. In this case the design matrix may be explored by the use of a combination of linearly or non-linearly (depending on the hypothesis relating to the relationship being investigated) varying contrast weights (e.g. 2 1 0 –1 –2) which sum to zero. This contrast vector may be applied across columns in the design matrix of a single subject, or across corresponding columns of different subjects in the same multi-subject design matrix. Alternatively, the weightings can be embodied within the design matrix itself, such that the first (or second or third etc.) order expansion of a boxcar or delta function (i.e. the zero order expansion) based on some known parameter (e.g. peak force of hand grip) is included as a column in the design

matrix. The variance in the observed data explained by this column over and above all the other columns is then calculated by weighting this column with +1, and all others with 0. Both of these approaches to parametric or correlation analyses have been used at least once in all experiments in this thesis.

Interactions and *factorial designs* allow one to investigate the effect of changing one parameter on the effect of a second parameter e.g. the effect of performing a motor task in the context of attending to the movement or not attending. By careful manipulation of the two parameters during an experiment, the main effects of motor performance and the main effects of attention can both be calculated, but so too can the effect of one on the other. This approach has not been used in this thesis, and so will not be discussed further.

If the factorial design allows one to look for the differences between differences, then it is also possible to examine for the similarities between differences or contrasts, by *conjunction analysis*. Conjunction analysis relies on the conjoint testing of multiple hypotheses. Thus for voxels which are significant in a conjunction analysis, it is possible to reject a number of null hypotheses simultaneously (Price and Friston, 1997). This approach has been used in chapter 5.

In summary, subspaces of the design space embodied in the design matrix may be interrogated by the use of contrasts. Specific experimental questions may be addressed either in the construction of the design matrix or in the construction of the contrast, or more usually both. It is this design that gives some biological meaning to the resulting t- or F- statistic.

3.8.3 Levels of inference

From the above description it can be seen how a result can be attributed to a specific cognitive or biological process. However, it is equally important to be clear about the statistical significance of a result.

An SPM will contain a number of voxels with a t- or F-statistic above a certain threshold. SPM derives p values pertaining to different levels of inference.

1. *Set-level inference* relates to whether the observed pattern of suprathreshold clusters is significant, but it is not clear whether single elements of this pattern are themselves significant, i.e. there is no power to localise a result to specific anatomical regions.
2. *Cluster-level inference* relates to whether a suprathreshold cluster is significant. Localising power is again sacrificed for statistical power, but the result may relate to a known anatomical region if the cluster is small enough.
3. *Voxel-level inference* is the most regionally specific, relating to whether the t- or F- test is significant at that voxel.

Both voxel- and cluster-level (but not set-level) inference, have been used in this thesis.

3.8.4 The problem of multiple comparisons

In a functional imaging experiment with no *a priori* hypothesis about the likely site of the expected signal change, inference is made at every voxel. The results across a whole brain volume are typically thresholded, so that only voxels in which the t- or F-statistic are above a certain value are considered. It is possible to

calculate the risk of rejecting the null hypothesis correctly (the p-value) for a given t- or F-statistic with known degrees of freedom. There are over 200 000 voxels in an fMRI experiment, and at the standard rejection rate of $p<0.05$, one would expect to reject the null hypothesis *by chance* in 10 000 voxels. In other words a false positive result will be observed in 5% of the voxels. This is clearly unsatisfactory, and typically one approach to the problem is to take the number of tests performed into account, and adjust (i.e. multiply) the p-value accordingly, to obtain an adjusted p-value. This is known as the Bonferroni correction. However, there is almost always some degree of spatial correlation in fMRI data. There are a number of reasons for this, but principally (i) the BOLD signal itself is smoothed in relation to the underlying neuronal activity and (ii) because of smoothing performed at the pre-processing stage and iii) because of correlations at a distance due to physiological correlations between voxels. Thus, the observations made in each voxel are not independent from those in neighbouring voxels, and so there are fewer independent observations in the brain volume overall. The Bonferroni correction is therefore too conservative for functional imaging data.

The approach taken in SPM is analogous to using the number of independent observations, rather than the number of voxels in a Bonferroni correction. However, it is not mathematically identical, primarily because the underlying smoothness of the observed data is not known, as the degree of spatial correlation prior to smoothing is also unknown. The smoothness of a statistical map can be estimated from the residual fields after normalisation by the variance at each voxel. Knowing the estimated smoothness, it is now possible to determine the number of *resels* (resolution elements) (Worsley *et al.*, 1992) in a statistical map.

The number of resels is dependent only on the smoothness of the data (which we have just estimated) and the number of voxels in the image. SPM uses random field theory (RFT), which defines the expected behaviour of smooth statistical maps to solve the problem. Any given thresholded statistical map will have an *Euler characteristic*, which provides a measure of the expected number of clusters above a given threshold. This information allows the calculation of the appropriate height threshold for a particular (adjusted) p-value, as the probability of type I error for a family of above threshold voxels is approximately equivalent to the expected Euler characteristic, E[*EC*]. For a two dimensional image,

$$\mathrm{E}[EC] = R(4\log_e 2)(2\pi)^{-3/2} Z e^{-1/2Z^2}$$

Where R is the number of resels, and Z is the Z-score threshold. In this way, it is possible to specify the threshold of Z-score above which the standard rejection rate of $p<0.05$ applies to any suprathreshold voxels. These principles apply equally to t- and F-random fields, and also to three-dimensional images.

3.9 Extending inference to populations: fixed and random effects analyses

So far we have been concerned with understanding the origin and analysis of a BOLD signal time series for a single voxel in the brain. SPM performs the same analysis in all voxels for a single subject study. In a group study, we now have data from multiple subjects at each voxel. The data from all subjects are concatenated into a single column vector, Y. In order to model the group data, a multiple subject design matrix is constructed (with a block diagonal structure, so

that subject specific effects are also modelled) and the same contrast vector that would be applied to the single subject design matrix is applied across all individual components of the multiple subject design matrix. SPM estimates the parameters of the model, and for a given contrast the t-statistic is dependent on the *within subject* variance. This gives a result that is effectively the group average and pertains only to those subjects in the study. This is termed a *fixed effects* approach.

However, it is likely that there will be some variability in responses between subjects, and this variability is not taken into account in a fixed effects analysis. If this variability is to be taken into account, then the effect of different subjects must be considered a random effect. In such a *random effects* analysis, the subjects can be viewed as having been drawn from a representative population. Therefore any results from a random effects analysis may be said to apply to that representative population.

Within SPM, random effects analysis may be undertaken using the *summary statistic* approach. For each subject the effect of interest is defined with a contrast vector. For each subject, a *contrast image* containing the contrast of the parameter estimates at each voxel is created and this is entered into a general linear model that implements a one-sample t-test. There are often fewer significant voxels in a random effects analysis precisely because the between subject variance dominates the result, but also because the degrees of freedom available are now dependent on the number of subjects rather than the number of scans (usually a very large number in fMRI experiments).

It is also possible to compare groups of subjects in order to make inferences about the differences between populations. In chapter 6, I compare single stroke patients with a group of healthy controls in such a two-sample t-test, in order to address the null hypothesis that there is no difference in task related brain activation in each patient compared to the normal population. It does not matter that the groups are of different sizes, because variance is assumed to be the same in the two groups, and so is estimated across all subjects in the two groups. All subjects must have identical design matrices at the first level analysis, i.e. the analysis that generates the contrast image, in order for the summary statistic approach to be valid.

Random effects analyses are not always suitable, and the approach in any given study must be dictated by the experimental question and whether it sees to make inferences about the study population or about the population at large.

Chapter 4

METHODS AND MATERIALS

II. SPECIAL CONSIDERATIONS

4.1 Introduction

Early experimental approaches using functional imaging to study stroke patients concentrated on patients with relatively good recovery. Consequently any differences in motor-related brain activations between stroke patients and normal subjects could not be specifically related to the recovery process. In order to examine for differential patterns of activation as a function of recovery, one must study patients with a broad range of recovery. This can be done across subjects with variable degrees of recovery, or longitudinally within subjects as they recover (figure 4.1). These approaches will be described in chapters 6, 7 and 8, and aim to answer two questions. Firstly, is the task-related activation pattern in an individual patient different to the normal population? Secondly, is there a correlation between the degree of recovery and the degree of task-related brain activation in some brain regions? This approach requires careful consideration of

(1) how recovery or outcome is measured, and (2) how to ensure that comparisons can be made between patients in terms of the task they are asked to perform during scanning session.

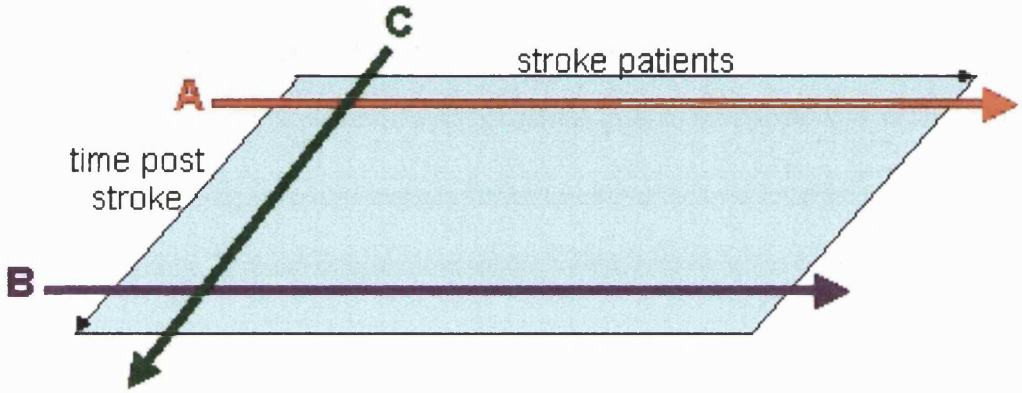


Figure 4.1 Schematic representing ways of correlating task-related brain activations with recovery, by cross-sectional studies in the (A) post-acute phase and (B) chronic phase, or (C) within patients longitudinally

4.2 Quantification of recovery

There are many outcome scores that reflect aspects of recovery, but not all are necessarily correlated with one another. The consensus view is that a range of measures should be used to build an overall picture of degree of recovery, (Duncan *et al.*, 2000; Turton and Fraser, 1986). This issue has been neglected by previous studies, so that patients have been described as fully recovered on the basis of a single measure. Combining a number of outcome measures in order to create a single representative measure of outcome (at the time of scanning) takes

account of the complex nature of the recovery process, but allows patients to be compared in terms of recovery or outcome.

4.2.1 Outcome scores

The following outcome scores were used in all the patient studies described. Details of how these tests are administered and scored are given in Appendix I.

4.2.1.1 Rankin Scale

The Rankin Scale claims to be a measure of handicap, but mixes impairment and disability. It has a strong emphasis on mobility. It is relatively insensitive but is very easy to use and extremely reliable (Van Swieten *et al.*, 1988).

4.2.1.2 Barthel Activities of Daily Living Index

The Barthel ADL index is the most widely used activities of daily living measure. It covers elements such as walking, dressing, going to the toilet and continence. Its validity and reliability have been demonstrated in many studies (Wade and Collin, 1989).

4.2.1.3 Orpington Prognostic Scale

The Orpington Prognostic Scale (OPS) is a stroke impairment scale, comparable to another well known scale, the National Institutes of Health Stroke Scale (NIHSS). Both have been found to be valid and reliable. The OPS is easier to use and more sensitive to change at higher levels of physical function (Lai *et al.*, 1998).

4.2.1.4 Motricity Index

The arm and leg sections of the Motricity Index were used in the assessments performed as part of this thesis. It is a short simple measure of motor loss with good validity and reliability (Sunderland *et al.*, 1989; Collen *et al.*, 1990).

4.2.1.5 Nine Hole Peg Test

The nine hole peg test (NHPT) is a simple and short test of manual dexterity. It is both valid and reliable (Mathiowetz *et al.*, 1985; Heller *et al.*, 1987; Sunderland *et al.*, 1989). It is particularly sensitive to changes in performance in the upper ranges, but is less useful in severe impairment as patients are not able to pick up the pegs. In addition it is not sensitive at detecting proximal weakness.

4.2.1.6 Grip strength

Measurement of maximum grip strength with the affected hand is a good prognostic indicator after stroke (Heller *et al.*, 1987). It has good validity and reliability and is sensitive to changes over a wide range of impairment (Sunderland *et al.*, 1989)

4.2.1.7 Action Research Arm Test

The Action Research Arm Test (ARAT) is a modification of a battery of tests first introduced in 1965 (Carol, 1965). It is designed to assess proximal and distal strength, as well as dexterity. The original test has been shortened (Lyle, 1981), but retains good validity and reliability.

4.2.1.8 Timed Ten-Metre Walk

Measuring the time it takes a subject to walk 10 metres is a simple, reliable, valid measure of mobility (Holden *et al.*, 1984; Wade *et al.*, 1987). It has been criticised for not considering the quality of the gait, but it is likely that good quality is associated with greater speed (Wade *et al.*, 1987).

The above measures cover a variety of aspects of the recovery process, including focal upper limb impairment (grip strength, and arm section of Motricity Index), focal upper limb disability (nine hole peg test), general upper limb ability (Action Research Arm Test), focal lower limb impairment (leg section of Motricity Index), mobility (timed ten metre walk, and to a certain extent the Rankin Scale), activities of daily living (Barthel ADL Index), some aspects of handicap (Rankin Scale) and a general stroke impairment score including a basic cognitive assessment (Orpington Prognostic Scale). The selection of a wide variety of outcome scores is made with a specific experimental question in mind. My intention is to investigate whether task (motor) related brain activation in stroke patients is related to their *overall* recovery, not specifically upper limb recovery. However, the lower limb tests are *not* independent of either ADL scores or upper limb scores. Walking speed for example is affected by both upper limb function and truncal stability and lower limb Motricity Index scores correlate with hand grip scores (Cameron and Bohannon, 2000) suggesting that processes that facilitate upper limb recovery also facilitate lower limb recovery.

These scores were used to provide a quantification of different aspects of the recovery process. In addition patients were also assessed for the presence of

sensory deficits, apraxia, and cognitive deficits, particularly neglect syndromes (see Appendix I).

4.2.2 Combining outcome scores: principal component analysis

Thus I scored a number of individuals on a variety of outcome measures, or individuals were scored at a number of time points. Several different measures were used, because no single measurement encompasses overall recovery. In order to determine how many trends are present in the data set I used a multivariate analysis technique called principal component analysis.

Suppose that p observations ($x_1, x_2, \ldots\ldots, x_p$) are made on each of n individuals. It is possible to combine these variables in the form of a number of different independent variables ($y_1, y_2, \ldots\ldots, y_p$). The new variables are defined,

$$y_1 = a_{11}x_1 + a_{12}x_2 + \ldots + a_{1p}x_p,$$

$$y_2 = a_{21}x_1 + a_{22}x_2 + \ldots + a_{2p}x_p.$$

The new variable y_1 explains the highest possible variance, y_2 the next largest variance, y_3 the third largest and so on. Each variable is uncorrelated, and so accounts for different trends in the original data set. Thus y_1 represents the linear combination of the xs that best explains the differences between the individual subjects (or time points), and is termed the first principal component.

The method involves calculating p eigenvalues of the covariance matrix such that,

$$\lambda_1 \geq \lambda_2 \geq \lambda_3 \geq \ldots \geq \lambda_p,$$

where, $$\lambda_i = \mathbf{var}(y_i).$$

The total variance in the original data set is preserved, such that,

$$\Sigma\ \mathbf{var}(x_i) = \Sigma\ \mathbf{var}(y_i) = \Sigma\ \lambda_i$$

Because alterations in the scale of the original variables will alter the result, it is important that the original variables are normalized (i.e. mean corrected and divided by the standard deviation) to give zero mean and unit variance. From the above equation it therefore follows that,

$$\lambda_1 + \lambda_2 + \lambda_3 + \ldots + \lambda_p = p.$$

Thus, the total variance of the original data set explained by the first principal component is λ_1 / p.

In summary, this process reparameterises the original data set, producing a new set of uncorrelated variables. Because the new variables have exactly the same total variance, principal component analysis is only useful if the majority of the variance is accounted for by a reduced number of new variables. I have used this method in the expectation that the first principal component will account for a large proportion of the variance and will be used to compare overall recovery or outcome between different patients, or across different time points in the same patient.

4.3 Choice of motor paradigm

4.3.1 Using a task that all patients can perform

Previous studies used finger tapping as the motor task, thus excluding patients who had not recovered fractionated finger movements. I chose isometric dynamic hand grip, because the ability to perform hand grip returns relatively early compared to fractionated finger movement (Heller *et al.*, 1987). This task is well suited for the study of previously paretic patients and those in whom recovery is less than complete. In addition impaired hand grip force compares well with other measures of upper limb function (Heller *et al.*, 1987; Sunderland *et al.*, 1989). The required rate of gripping and feedback of force exerted were provided visually to subjects in the scanner. Thus patients with poor outcome were able to perform the task so I was not reliant on selecting only patients who could perform fractionated finger movements.

4.3.2 Avoiding the confound of differential task difficulty across subjects

The network subserving motor performance will be differentially engaged depending not only on the task used, but also on the level of difficulty of that task. Any differences in functional imaging results detected in patients compared to normals, or indeed across patients with different degrees of recovery, could be attributed to either differences in neuronal or cognitive reorganisation (Price and Friston, 1999). In studies where patients (with impaired motor performance) are asked to perform a motor task with the same absolute parameters (e.g. rate of finger tapping) as normal controls, patients will find the task more effortful or cognitively complex. Differences in activation in the patient group may then be

attributable to neuronal reorganisation (often referred to as plasticity) or differences in the effort exerted. For example, complex motor tasks are known to recruit a wider network of motor regions than simple motor task (Catalan *et al.*, 1998). Thus, to control as much as possible for the degree of effort involved in performing a motor task, performance levels should be maintained constant across all subjects (controls and patients). In the case of the isometric dynamic hand grip task this is achieved by setting a target force and rate of hand grips differently for each subject, i.e. as a constant proportion of each subject's own maximum performance parameters at the time of scanning. By performing a motor task (hand grip) that is more reflective of intrinsic motor recovery than adaptation (Sunderland *et al.*, 1989) and controlling for motor effort as much as possible, the activations within known motor-related regions are more likely to reflect neuronal reorganisation.

4.4 Summary

The main experimental aim of this thesis is to explore the relationship between the recovery process and altered patterns of brain activation after stroke. My experimental design must address two neglected areas. The first is how to measure a complex process such as functional recovery. The second is how to ensure that comparisons can be made between patients in terms of the task they are asked to perform during scanning session. I have approached the first issue by employing a range of outcome scores, and have used the technique of principal component analysis to extract the main trends from these data sets. The second issue will be addressed by manipulating the parameters of the motor task separately for each

subject in order to control for effort across subjects. These approaches are novel and will allow inferences about the relationship between recovery and brain activation to be made.

Chapter 5

THE FUNCTIONAL ANATOMY OF HAND GRIP

5.1 Introduction

Hand grip is a motor task commonly employed in the real world. However, it has been used relatively infrequently in functional imaging experiments for the purposes of studying the cortical and subcortical correlates of the motor system in humans. This chapter deals with the characterisation of the functional anatomy of isometric dynamic hand grips in normal subjects. This will enable comparisons to be made with brain activation patterns evoked by the same task in stroke patients. The rationale for choosing hand grip as the motor task in stroke patients was discussed in chapter 4.

There are two aspects of this characterisation that are of particular interest. Firstly, the relationship between changes in the BOLD signal and changes in peak hand grip force, and secondly the effects of changing age on task related brain activation patterns.

5.1.1 The effects of changing peak hand grip force

Studies in humans are limited. Dettmers *et al.*, (1995) demonstrated a linear relationship between increasing regional cerebral blood flow (rCBF) and increasing force generated by a finger tapping task in a number of motor regions. Thickbroom *et al.*, (1999) did not find a relationship between size of activation and force exerted in a dynamic hand grip task. However, using single cell recording in macaque monkeys, the correlation between firing rates and force of both isometric and dynamic grip tasks has been demonstrated to be mainly monotonic or linear for many years (Ashe, 1997). The observation of non-linear changes in firing rates of single cortical cells has also been made for a number of regions (Cheney and Fetz, 1980; Evarts *et al.*, 1983) although with less consistency. By performing both a parametric analysis using linear and non-linear regressors, I examined for both linear and non-linear effects of increasing force across the network of regions activated by hand grip.

5.1.2 The effects of age

The stroke patients studied in this thesis come from a wide range of age groups though are more frequently elderly. It is therefore imperative to investigate the effects of ageing on the neural correlates of the hand grip paradigm.

Impairments in a number of cognitive tasks are seen as part of the normal ageing process in humans. Functional imaging studies have examined differences in recruitment of brain regions during both motor (Calautti *et al.*, 2001c; Mattay *et al.*, 2002) and cognitive tasks (D'Esposito *et al.*, 1999; Grady *et al.*, 2000; Esposito *et al.*, 1999). Many of these studies have found greater activations in

older compared to younger subjects in a number of brain regions. However, this may only be the case for those elder subjects in whom levels of performance are comparable to those in younger subjects (Mattay *et al.*, 2002; Reuter-Lorenz *et al.*, 1999). It has been suggested that interruption of the normal neural networks subserving cognitive performance by age related neurodegenerative and neurochemical changes underlies a decline in function (Volkow *et al.*, 1998; Wenk *et al.*, 1989), but that compensatory processes in cortical and subcortical function allow maintenance of performance level in some elderly people.

Linear decreases in performance as a function of increasing age have been demonstrated with motor tasks such as repetitive finger tapping (Shimoyama *et al.*, 1990). More complex, non-linear effects are seen in more demanding timed tasks and visually guided hand movements (Kauranen *et al.*, 1996; Houx *et al.*, 1993; Smith *et al.*, 1999). Functional imaging studies of changes in the motor system have used repetitive or cued finger tapping (Calautti *et al.*, 2001c; Mattay *et al.*, 2002) and have shown greater age-associated activation in a number of brain regions within the motor system. I hypothesized that isometric dynamic hand grip would result in activations in a widespread fronto-parietal network (Binkofski *et al.*, 1998; Ehrsson *et al.*, 2001), within which I would demonstrate age related differential activations. Furthermore, it is likely that any age effects will be non-linear, in view of the degree of 'on-line' monitoring and precision required during the hand grip task (Smith *et al.*, 1999).

5.2 Materials and methods

5.2.1 Subjects

Twenty six healthy volunteers, aged 21-80 years, comprising seventeen male subjects (range 27 to 80 years, mean age 50.2 years) and nine female subjects (range 26 to 66, mean age 44.7), participated in the study. One subject performed tasks only with the dominant hand, and one subject only with the non-dominant hand. All subjects were right handed according to the Edinburgh handedness scale (Oldfield, 1971). They reported no history of neurological illness or psychiatric history and were not taking regular medication. Full written consent was obtained from all subjects in accordance to the Declaration of Helsinki. The study was approved by the Joint Ethics Committee of the Institute of Neurology and National Hospital for Neurology and Neurosurgery, London.

5.2.2 Motor paradigm

Subjects performed a dynamic isometric hand grip task using dominant (right) and non-dominant (left) hands in separate sessions and in a randomised counterbalanced order, using a magnetic resonance imaging compatible manipulandum consisting of two force transducers (Honeywell FSG15N1A, Honeywell, NJ, USA) situated between two moulded plastic bars (figure 5.1). Compression of the two bars by isometric handgrip resulted in the generation of a differential voltage signal, linearly proportional to force exerted, which was fed into a signal conditioner (CED 1902, Cambridge Electronic Design, Cambridge, UK). This signal was digitised (CED 1401, Cambridge Electronic Design, Cambridge, UK) and fed into a computer running Cogent 2000 (Wellcome

Department of Imaging Neuroscience http://www.fil.ion.ucl.ac.uk/Cogent2000.html). The dynamic change in recorded signal was projected in real time onto a screen as a column whose height varied linearly with change in voltage and hence force.

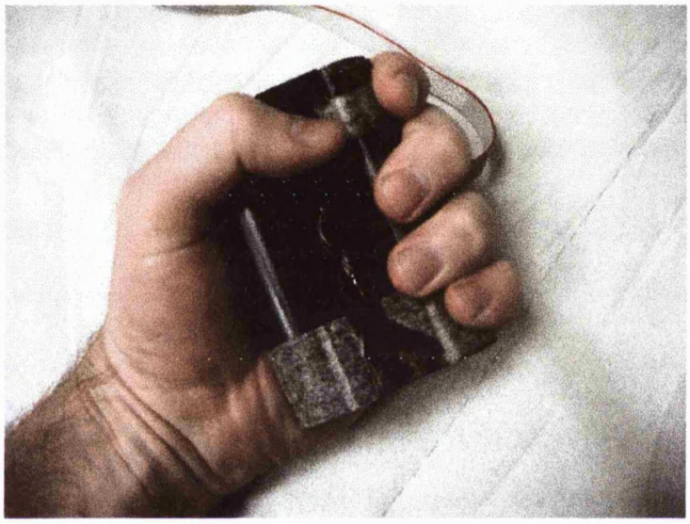


Figure 5.1 Hand grip manipulandum used in all experiments

Prior to scanning, but whilst lying in the scanner, subjects were asked to grip the manipulandum with maximum force to generate a maximum voluntary contraction (MVC). During scanning, subjects performed paced isometric dynamic handgrips in blocks of 20 seconds, alternating with 20 seconds rest. A total of twenty four blocks of handgrip, and twenty four rest blocks were performed per session. Target forces and rates of handgrip were constant within each twenty second block, but were varied between blocks, in a randomised counterbalanced order. Target forces during scanning were set at 10%, 20%, 40% and 60% of MVC for each subject indicated by a horizontal bar on the screen. The

required rate of hand grip was indicated visually by a cross displayed at the bottom of the screen for 0.3 seconds at a rate of either 0.33 Hz or 0.67 Hz. Both rates of grip were comfortable for all subjects. Variation in rate was included only to help maintain subjects' attention throughout a session. The appearance of the cross indicated that the subject was to perform a single brief handgrip, to be continued until the column representing force applied came into contact with the horizontal bar on the screen, at which point the grip could be released. Prior to scanning, subjects were pre-trained on at least eight handgrip-rest blocks, or until comfortable with the task utilising each of the eight force-rate combinations.

5.2.3 Data acquisition

A Siemens VISION system (Siemens, Erlangen, Germany), operating at 2 T, was used to acquire both T_1-weighted anatomical images (1 x 1 x 1.5 mm voxels) and T_2*-weighted MRI transverse echo-planar images (EPI) (64 x 64 3 x 3 mm^2 pixels, T_E = 40ms) with blood oxygenation level dependent (BOLD) contrast. Each echoplanar image comprised forty eight 1.8 mm thick axial slices taken every 3mm, positioned to cover the whole cerebrum. A total of 270 volumes were acquired continuously during each session, with an effective repetition time (T_R) of 3.649 seconds per volume. The first six volumes were discarded to allow for T_1 equilibration effects.

5.2.4 Image analysis

Imaging data were analysed using Statistical Parametric Mapping (SPM99, Wellcome Department of Imaging Neuroscience,

http://www.fil.ion.ucl.ac.uk/spm/) (Friston *et al.*, 1995a; Worsley and Friston 1995) implemented in Matlab5 (The Mathworks Inc., USA). Image pre-processing was carried out as described in chapter 3. Part of the experiment was to compare activation patterns obtained using the dominant and non-dominant hands. In order to make this direct comparison (by flipping some images about the midsagittal line), a separate set of images was normalized using a symmetrical EPI template. This was created by averaging the standard EPI template and its mirror image about the midsagittal plane. All normalised images were then smoothed with an isotropic 8 mm full-width half-maximum Gaussian kernel. The time series in each voxel were high pass filtered at 1/100 Hz to remove low frequency confounds and scaled to a grand mean of 100 over voxels and scans within each session.

Statistical analysis was performed in two stages. In the first stage, using a single subject fixed effects model, all handgrips were defined as a single event type and modelled as delta functions (Friston *et al.*, 1998). The data were modelled using a set of orthogonalised polynomial expansions up to the third order (by forward model selection). Each term is represented by the interaction between a delta function and the peak force exerted (expressed as a percentage of MVC) during each handgrip. In general, the n^{th} order term is given by $force^n \cdot delta$ (Büchel *et al.*, 1998) (figure 5.2).

The resulting covariates were convolved with a canonical synthetic haemodynamic response function, and were used in a general linear model (Friston *et al.*, 1995a) together with a single covariate representing the mean (constant) term over scans. The parameter estimates for each covariate resulting from the least mean squares fit of the model to the data were calculated, and

statistical parametric maps of the t statistic (SPM{t}) resulting from linear contrasts of each covariate (Friston *et al.*, 1995a) were generated and stored as separate images for each subject.

In order to create activation maps representing the main effects of hand grip (0^{th} order), as well as linear (1^{st} order), and non-linear (2^{nd} and 3^{rd} order) changes of signal in relation to peak hand grip force, random-effects analyses were performed (Friston *et al.*, 1999). 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 one sample t-tests for each covariate of interest. The SPM{t}s were thresholded at $p < 0.05$, corrected for multiple comparisons across whole brain.

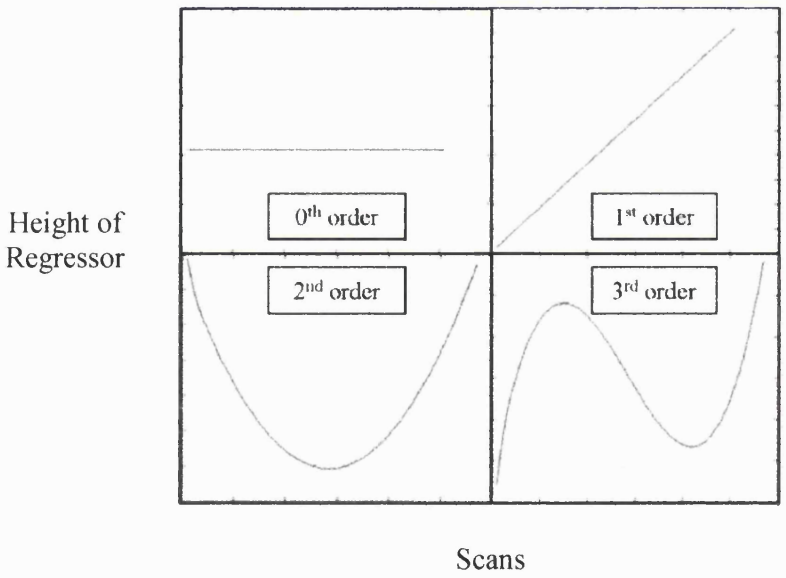


Figure 5.2 The concept of orthogonalised polynomial regressors is illustrated using these simple examples. The 0^{th} order term represents the height of the delta functions used to model each event. The 1^{st}, 2^{nd}, and 3^{rd} order terms represent the element-wise product of a linear (or non-linear) term and the 0^{th} order term (Büchel *et al.*, 1998). The orthogonalised 1^{st}, 2^{nd}, and 3^{rd} order terms are shown.

I was interested to make direct comparisons between task related brain activations using the dominant and non-dominant hands. Contrast images for the main effects of hand grip for the dominant and non-dominant hands were entered into a two sample t-test. By entering two contrast images per subject into the model, a potential source of non-sphericity was introduced. Sphericity refers to the assumption of identically and independently distributed errors. Non-sphericity correction within SPM was therefore applied. Weighted least squares approach provides maximum likelihood estimators based on the non-sphericity. In this case the weighting 'whitens' the errors rendering them identical and independently distributed.

The comparison between hands was performed in three stages; (1) a direct comparison of the contrast images for dominant and non-dominant hand grip, (2) a comparison of contrast images flipped and unflipped about the sagittal midline for each hand, (3) a comparison of dominant hand unflipped contrast images, and flipped non-dominant hand contrast images. SPM{t}s were thresholded at $p < 0.05$, corrected for multiple comparisons across whole brain.

An additional experimental question concerned the possible effect of age on both the main effects of hand grip, and on the linear and non-linear effects of increasing force. Thus, I performed linear regression analyses within SPM99, in which the two orthogonal covariates were (1) contrast images for each subject for the effect of interest (0^{th}, 1^{st}, 2^{nd}, or 3^{rd} order effects), and (2) a single value representing age^2 for each subject (mean corrected across the group). I hypothesised *a priori* that I would find non-linear changes in activation in keeping with previous behavioural data (Smith *et al.*, 1999), and so chose to use age^2

rather than age as the second covariate. In order to identify differential age related effects only within the motor network activated by hand grip I performed a conjunction analysis between two orthogonal contrasts, effects of hand grip and effects of age^2. Conjunction analysis relies on the conjoint testing of multiple, in this case two, hypotheses. Thus for voxels that are significant in the conjunction analysis I was able to reject the null hypotheses that there is no effect of age^2 AND no effect of hand grip, i.e. significant voxels exhibit a response to hand grip, and this response varies as a function of age^2 (Price and Friston, 1997). It is possible to perform such a conjunction analysis at the random effects level, because only one contrast image per subject is entered into the model so that the covariates are independent and orthogonal, thus avoiding assumptions about sphericity. For significant voxels, the correlation coefficient for the plot of parameter estimate against age^2 for each subject, together with the corresponding *p*-value, was calculated.

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 standard MNI brain and on the normalised structural images of each subject, and labeling with the aid of the atlas of Duvernoy (1999).

5.3 Results

5.3.1 Behavioural results

There was no difference in mean MVC exerted by the dominant hand compared to the non-dominant hand. Neither was there a correlation between age^2 and peak MVC (dominant hand, $r^2 = 0.06$, p= not significant; non-dominant hand $r^2 = 0.01$, p= not significant).

5.3.2 Main effects of handgrip

Regions activated in the main effect comparison of isometric dynamic hand grip compared to rest, irrespective of force applied, are shown in table 5.1 and figure 5.3. Activations were seen in a network of regions, which was similar for dominant and non-dominant hands. The most lateralised activations were in contralateral sensorimotor cortex and ipsilateral superior cerebellum. Other activations were bilaterally distributed, including dorsal lateral premotor cortex (PMd) and ventral lateral premotor cortex (PMv), supplementary motor area (SMA), cingulate motor areas, inferior parietal cortex and intraparietal sulcus, insula cortex, cerebellar vermis, and both inferior and superior cerebellar hemispheres.

Table 5.1 *Main effects of hand grip*

	Dominant Hand					Non-dominant Hand				
		Talairach coordinates in MNI space					Talairach coordinates in MNI space			
Region	Side	x	y	z	Z-value	Side	x	y	z	Z-value
central sulcus	CL	-34	-30	56	7.38	CL	38	-26	52	7.1
	CL	-30	-30	66	6.4					
postcentral gyrus	CL	-52	-24	32	6.32	CL	54	-18	50	6.46
	CL	-54	-18	26	6.37	CL	48	-26	58	6.31
postcentral sulcus	CL	-50	-22	50	6.61	CL	54	-20	38	5.7
precentral gyrus (BA 4/6)						CL	36	-22	64	7.31
PMd	CL	-34	-6	58	6.33	CL	42	-10	54	5.87
	CL	-24	-12	66	6.11	IL	-38	-4	52	5.76
	IL	28	0	54	5.94					
	IL	40	2	60	5.15					
caudal PMv	CL	-54	6	6	5.85	IL	-54	6	6	5.05
	IL	50	6	28	6.6	IL	-58	10	26	5.34
rostral PMv (BA 44)	CL	-56	6	14	5.2	CL	62	16	12	6.95
	IL	58	16	12	5.74	IL	-58	6	4	5.05
rostral cingulate sulcus	CL	-10	10	40	6.72					
	IL	2	2	50	6.48					
caudal cingulate sulcus	CL	-6	-4	54	6.39	IL	-10	-6	62	5.67
SMA	CL	-8	-4	64	6.16	CL	8	-2	68	6.31
pre-SMA	IL	10	2	66	6.61	CL	8	4	58	6.28
insula cortex	CL	-38	2	-2	6.3	CL	40	4	2	6.25
	CL	-40	14	-4	5.53	CL	50	14	-8	5.85
	IL	40	8	-4	6.54	IL	-36	12	-2	6.07
	IL	44	2	10	5.55	IL	-38	4	0	6.06
supramarginal gyrus	CL	-44	-36	24	6.12	IL	-52	-26	36	5.65
	IL	52	-36	44	5.98					
intraparietal sulcus	IL	48	-38	52	6.15	CL	30	-78	26	5.47
	IL	28	-76	26	5.96	IL	-32	-52	54	6.35
superior parietal cortex	CL	-30	-54	58	6.05					
parietal operculum	CL	-46	-26	16	5.88	CL	56	-18	22	6.16
frontal operculum	CL	-46	0	6	6					
superior temporal gyrus	CL	-48	8	-10	5.72	CL	64	-30	20	5.48
putamen	CL	-22	-4	2	6.3	CL	28	4	-2	6.14
						IL	-22	0	6	5.49

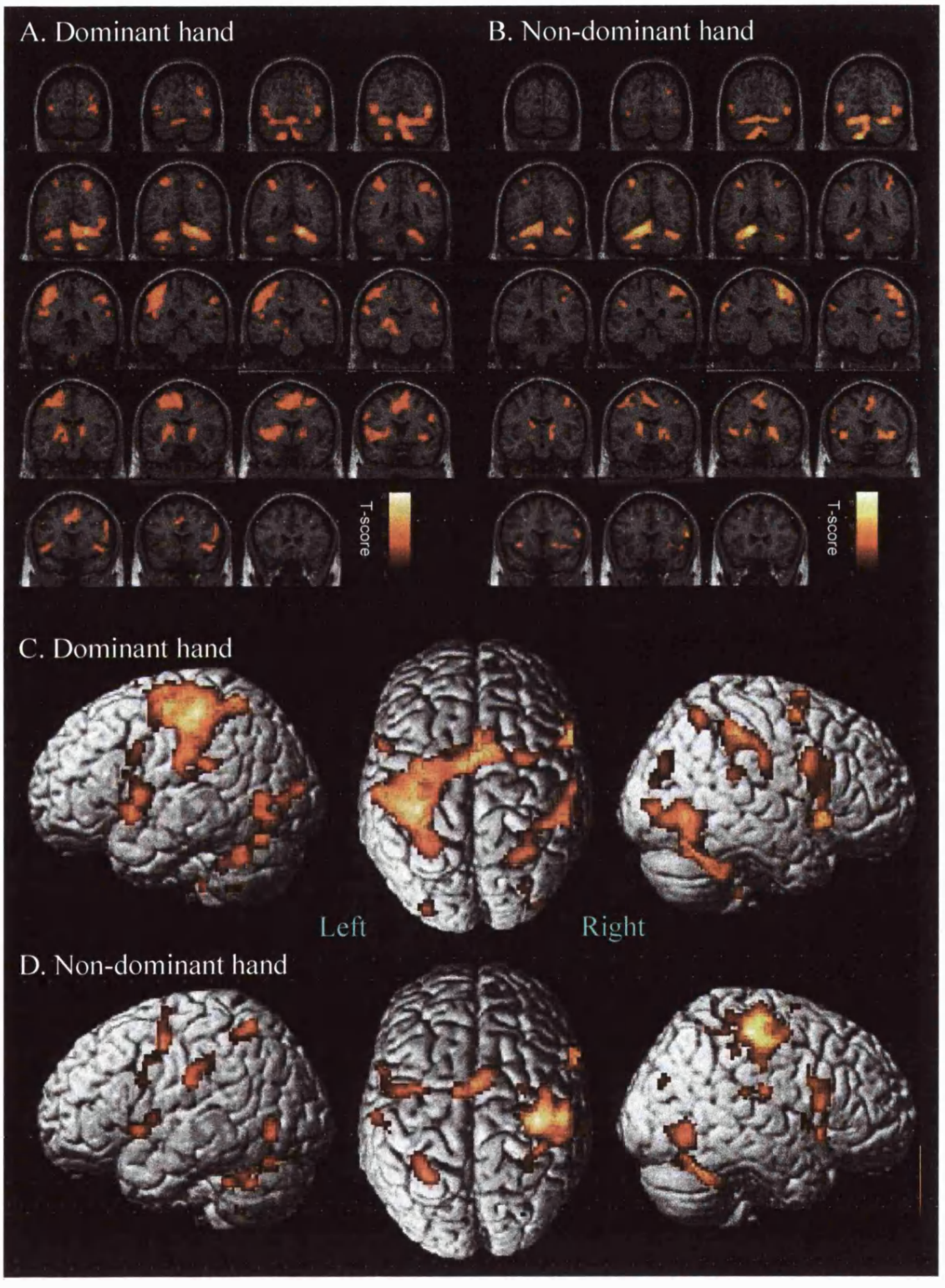
Table 5.1 continued

ventrolateral thalamus	CL	-14	-16	2	6.05					
	IL	16	-10	12	5.87					
posterior lateral thalamus						CL	18	-18	14	5.48
red nucleus	CL	-12	-14	-6	6.06					
cerebellum (V)	IL	20	-50	-20	> 8.0	IL	-14	-50	-22	7.83
cerebellum (VI)	IL	38	-50	-32	6.57	IL	-12	-70	-22	6.09
cerebellum (VI)	CL	-30	-54	-30	7.05	CL	28	-66	-24	6.88
cerebellum (VI)	CL	-28	-66	-20	6.68	CL	34	-58	-26	6.31
cerebellum (CrI)	CL	-36	-44	-40	5.42	IL	-46	-58	-34	6
						CL	46	-56	-30	5.66
cerebellum (VIIIA)	CL	-20	-68	-46	6.95	CL	12	-72	-42	5.36
cerebellum (IX)	IL	12	-60	-48	7.65	IL	-14	-56	-48	7.2
cerebellar vermis (V)	M	6	-54	-18	7.51					
cerebellar vermis (VI)	M	6	-66	-20	7.05	M	-4	-68	-24	6.88
cerebellar vermis (VIIIB)						M	-4	-70	-36	6.42

Location of brain regions demonstrating significant activations for single handgrip compared to rest given as MNI coordinates (x, y, z) in Talairach space. All voxels significant at threshold of $p < 0.05$, corrected for multiple comparisons across whole brain. SMA = supplementary motor area; PMd = dorsal lateral premotor cortex; PMv = ventral lateral premotor cortex; BA = Brodmans areea; CL = contralateral; IL = ipsilateral, M = midline.

Legend to figure 5.3 SPM{t}s representing the main effects of hand grip for (A and C) dominant hand, and (B and D) non-dominant hand compared to rest, obtained in random effects one sample t-test analysis. In A and B, results are displayed on a canonical T1-W MRI in coronal sections 6mm apart. In C and D, results are rendered onto canonical brain. The brain is shown (from left to right) from the left side, from above (left hemisphere on the left), and from the right. All voxels are significant at $p < 0.05$, corrected for multiple comparisons across whole brain.

Figure 5.3


A. Dominant hand
B. Non-dominant hand
T-score
T-score
C. Dominant hand
Left
Right
D. Non-dominant hand

I was also interested to make comparisons between task related brain activations using the dominant and non-dominant hands, by comparing flipped and unflipped, dominant and non-dominant hand contrast images (table 5.2). Categorical comparisons of dominant and non-dominant hand grip demonstrated that the activation pattern generated by each hand differed from the other only by the activation of contralateral sensorimotor cortex and ipsilateral superior cerebellum (figure 5.4a and 5.4b). Secondly, comparison of flipped and unflipped contrast images for dominant hand, suggested that the only lateralised task related activations were in contralateral sensorimotor cortex, frontal operculum, and ipsilateral cerebellum (Figure 5.4c). The same comparison for non-dominant hand demonstrated that lateralised task related activations were seen only in contralateral sensorimotor cortex, angular gyrus, and ipsilateral cerebellum (figure 5.4d). Lastly, the comparison of unflipped dominant to flipped non-dominant hand contrast images, demonstrated increased activation for dominant hand in contralateral parietal operculum and insula cortex, and ipsilateral intraparietal sulcus and posterior inferior frontal gyrus (BA 44/45) (figure 5.4e).

There were no voxels more active during rest than dominant hand grip, but regions within anterior cingulate cortex were more active during rest than non-dominant hand grip (peak voxel at x = 0, y = 34, z = -16, Z-score = 5.76). Non-significant decreases in BOLD signal were seen in ipsilateral M1 during use of either hand.

Table 5.2 *Comparison of dominant and non-dominant hands*

Region	Side	Talairach coordinates in MNI space x	y	z	Z-value
(a) dominant hand vs non-dominant hand					
sensorimotor cortex	L	-34	-30	58	> 8.0
cerebellum (V)	R	18	-50	-22	> 8.0
cerebellum (XI)	R	12	-62	-48	5.86
(b) non-dominant hand vs dominant hand					
sensorimotor cortex	R	38	-24	50	> 8.0
cerebellum (V)	L	-14	-50	-22	> 8.0
(c) dominant hand vs flipped dominant hand					
sensorimotor cortex	L	-34	-28	56	> 8.0
cerebellum (V)	R	16	-48	-22	> 8.0
cerebellum (XI)	R	12	-60	-50	6.12
frontal operculum	L	-42	-4	6	5.53
(d) non-dominant hand vs flipped non-dominant hand					
sensorimotor cortex	R	38	-26	54	> 8.0
cerebellum	L	-14	-50	-22	> 8.0
angular gyrus	R	32	-76	28	5.08
(e) dominant handgrip vs flipped non-dominant handgrip					
parietal operculum	L	-42	-38	24	6
insula cortex	L	-42	-4	6	5.58
intraparietal sulcus	R	32	-76	28	5.49
posterior inferior frontal gyrus	R	56	20	26	5.46

Location of brain regions representing differences between the main effects of dominant and non-dominant hand grip, given as MNI coordinates (x, y, z) in Talairach space. Flipped images are those rotated about the midsagittal plane. All voxels significant at threshold of $p < 0.05$, corrected for multiple comparisons across whole brain. R = right; L = left.

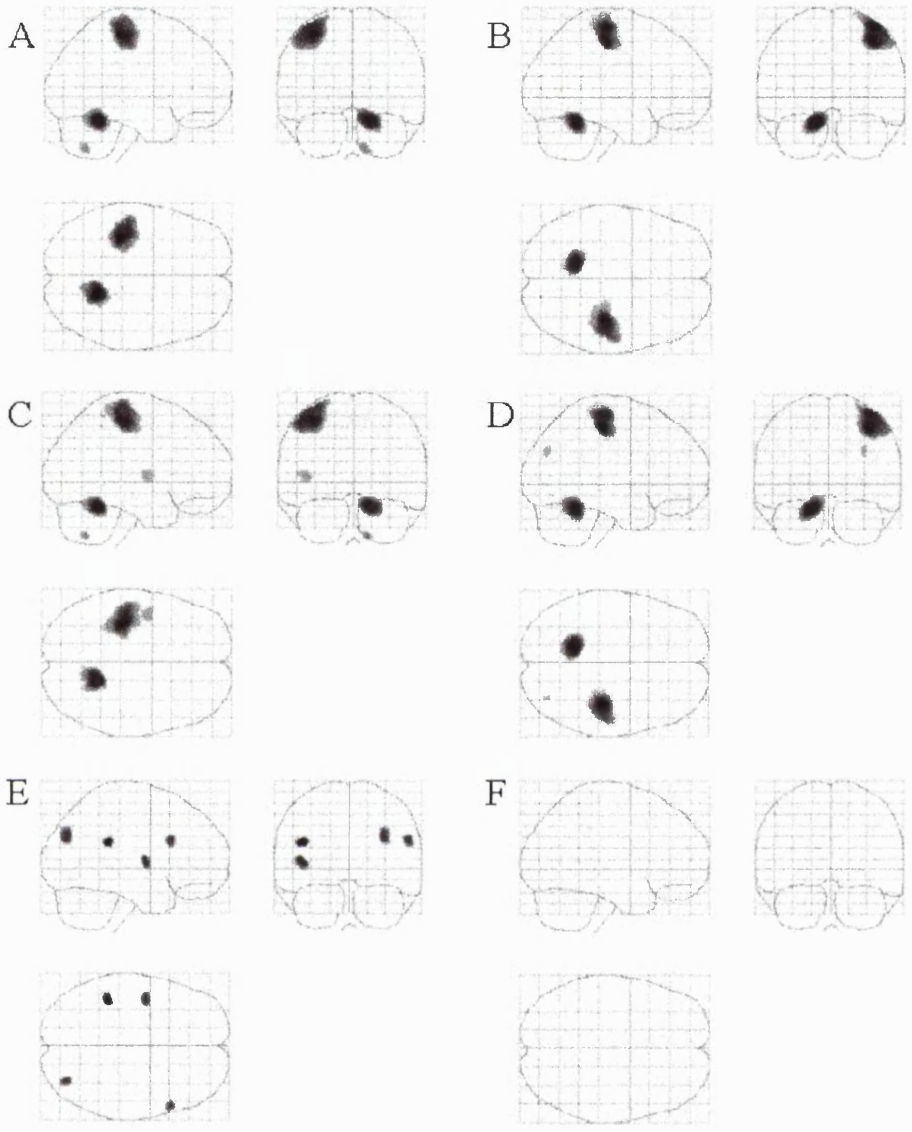


Figure 5.4 SPM{t}s representing the categorical comparison of the main effects of dominant and non-dominant hand grip, obtained in random effects two sample t-test analysis. Where images are 'flipped', this refers to flipping about the midsagittal plane (see METHODS section for details). (A) Dominant versus non-dominant hand grip, (B) non-dominant versus dominant hand grip, (C) dominant hand grip versus flipped dominant hand grip, (D) non-dominant hand grip versus flipped non-dominant hand grip, (E) dominant hand grip versus flipped non-dominant hand grip, (F) flipped non-dominant hand grip versus dominant hand grip. The SPM{Z}'s are shown as maximum intensity projections. The brain is shown from the right, top, and back. All voxels are significant at $p < 0.05$, corrected for multiple comparisons across whole brain.

5.3.3 Linear correlations between bold signal and force of handgrip

The statistical parametric map derived from the parameter estimates for the first order polynomial expansion of the handgrip force represents voxels in which the BOLD signal is linearly related to force of hand grip. These regions are shown in table 5.3 and figure 5.5. Linear increases in BOLD signal as a function of increasing grip force with either hand were seen in contralateral sensorimotor cortex, cerebellar vermis, ipsilateral cerebellar hemisphere (inferior cerebellum during dominant hand grip, superior cerebellum for non-dominant hand grip), ipsilateral ventroposterior lateral thalamus. In addition, linear correlations between BOLD signal and force of handgrip were seen in caudal cingulate sulcus with dominant hand grip, and contralateral ventral posterolateral thalamus with non-dominant hand grip.

Table 5.3 *Regions exhibiting a linear relationship between BOLD signal and peak force of hand grip force-related*

Region	Talairach coordinates in MNI space x	y	z	Z-value
(a) Right (dominant) hand grip				
Left sensorimotor cortex	-36	-36	60	5.15**
	-34	-28	64	5.12**
Right inferior cerebellum	8	-66	-42	5.17**
Cerebellar vermis	4	-42	0	4.05‡
Right thalamus (vpl)	20	-26	6	4.41‡
Left caudal cingulate sulcus	-6	-12	48	3.63‡
(b) Left (non-dominant) hand grip				
Right sensorimotor cortex	34	-22	52	4.96**
Cerebellar vermis	-4	-52	-12	4.78‡
Left superior cerebellum	-10	-50	-20	4.44*
Left thalamus (vpl)	-18	-16	16	4.44‡
Right thalamus (vpl)	12	-16	16	4.24*

Location of brain regions in which increasing BOLD signal was linearly related to peak force of hand grip, given as MNI coordinates (*x, y, z*) in Talairach space.
** = voxel significant at $p < 0.05$ after correction for multiple comparisons across whole brain. *= peak voxel within a cluster which is significant at $p < 0.05$ after correction for multiple comparisons across whole brain. ‡= voxel significant at $p < 0.05$ after correction for multiple comparisons across predefined volume of interest (VOI). For right hand grip, VOI defined by spheres of 10mm radius centred on the following coordinates, derived from Dettmers *et al.* (1995), left sensorimotor area (x=-20, y=-30, z=64), left cingulate motor area (x=-2, y=-14, z=48), right thalamus (x=18, y=-18, z=8), cerebellar vermis (x=4, y=-44, z=-8). For left hand grip, corresponding co-ordinates in the opposite hemisphere were used.

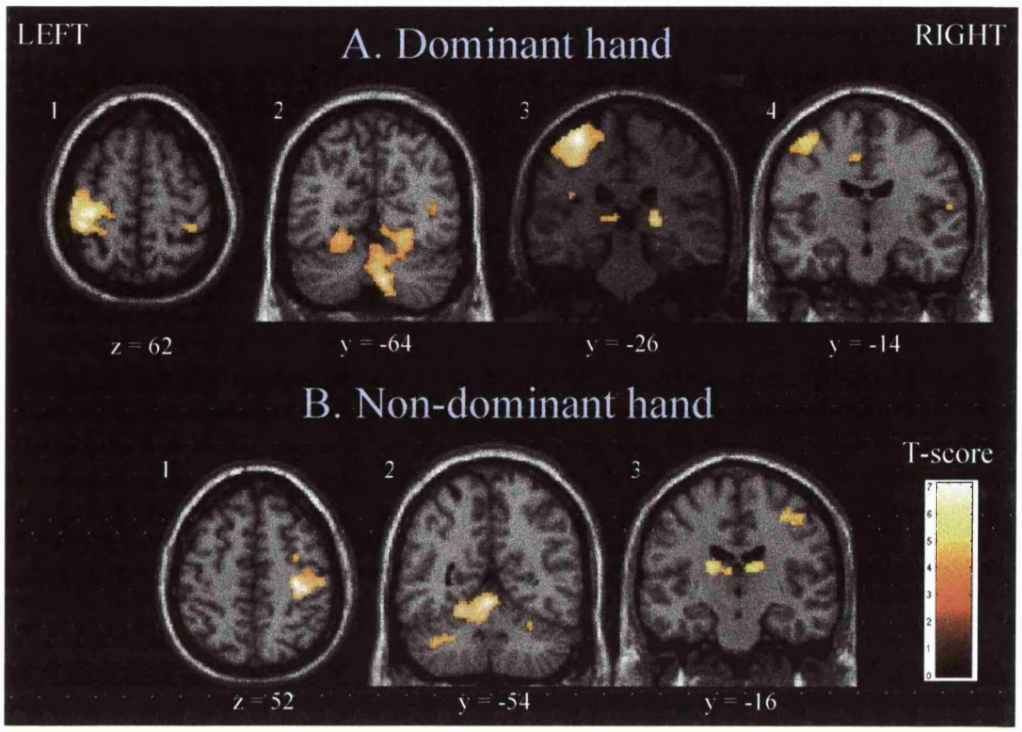


Figure 5.5 Regions in which there is a significant linear (1st order) relationship between BOLD signal and increasing hand grip force for (A) dominant hand, and (B) non-dominant hand. Results are displayed on a canonical T1-W MRI. All clusters are significant at $p < 0.05$, corrected for multiple comparisons across whole brain.

A.1 left sensorimotor cortex

A.2 right inferior cerebellum

A.3 left sensorimotor cortex and right ventral posterolateral thalamus

A.4 left sensorimotor cortex and left caudal cingulate sulcus

B.1 right sensorimotor cortex

B.2 left superior cerebellum and vermis

B.3 right sensorimotor cortex and bilateral ventral posterolateral thalami

5.3.4 Non-linear correlations between bold signal and force of handgrip

The statistical parametric maps derived from the parameter estimates for the second or third order polynomial expansion of the handgrip force represent voxels in which the BOLD signal exhibits a non-linear relationship to force of hand grip (see figure 5.2). No such relationship was consistently demonstrated with use of the non-dominant hand. Both positive and negative second order effects were seen with dominant hand use (table 5.4). Positive second order effects were seen in contralateral rostral cingulate sulcus and intraparietal sulcus, ipsilateral superior frontal sulcus, caudal cingulate sulcus and dorsolateral prefrontal cortex and bilaterally in insula cortex (figure 5.6A). Ipsilateral intraparietal sulcus exhibited a non-significant trend ($p=0.065$, corrected for multiple comparisons across whole brain, for a cluster with the peak voxel at x=44 y=-42 z=52). A negative second order effect was seen in a cluster with peak voxel in right medial orbitofrontal cortex (figure 5.6B). The cluster of activated voxels extended from x = -20 to x = +10. No areas demonstrating significant positive third order effects were seen, but negative third order effects were seen in contralateral superior cerebellum, ipsilateral insula cortex and ipsilateral frontal operculum (figure 5.6C).

Table 5.4 *Non-linear force-related regions for dominant hand grip*

Region of activation	Talairach coordinates in MNI space x	y	z	Z-score
(a) 2nd order (+)				
Right superior frontal sulcus	24	6	68	4.61
Left insula cortex	-42	18	2	4.49
Right caudal cingulate sulcus	4	-4	40	4.23
Left rostral cingulated sulcus	-6	14	36	4.04
Right DLPFC	32	52	26	3.95
Left intraparietal sulcus	-36	-36	42	3.81
Right insula cortex	34	14	-2	3.74
(b) 2nd order (-)				
Right medial orbitofrontal cortex	4	46	-20	4.44
(c) 3rd order (+)				
No significant voxels or clusters at group level				
(d) 3rd order (-)				
Left superior cerebellum	-32	-60	-30	4.45
Right insula cortex	46	16	-6	3.71
Right frontal operculum	50	28	-4	3.76

Location of brain regions in which there is a non-linear relationship between BOLD signal and force applied during a single dominant hand grip are given as MNI coordinates (*x*, *y*, *z*) in Talairach space. Clusters are significant at $p < 0.05$ after correction for multiple comparisons across whole brain, and the peak voxel within each cluster is reported.
DLPFC = dorsolateral prefrontal cortex.

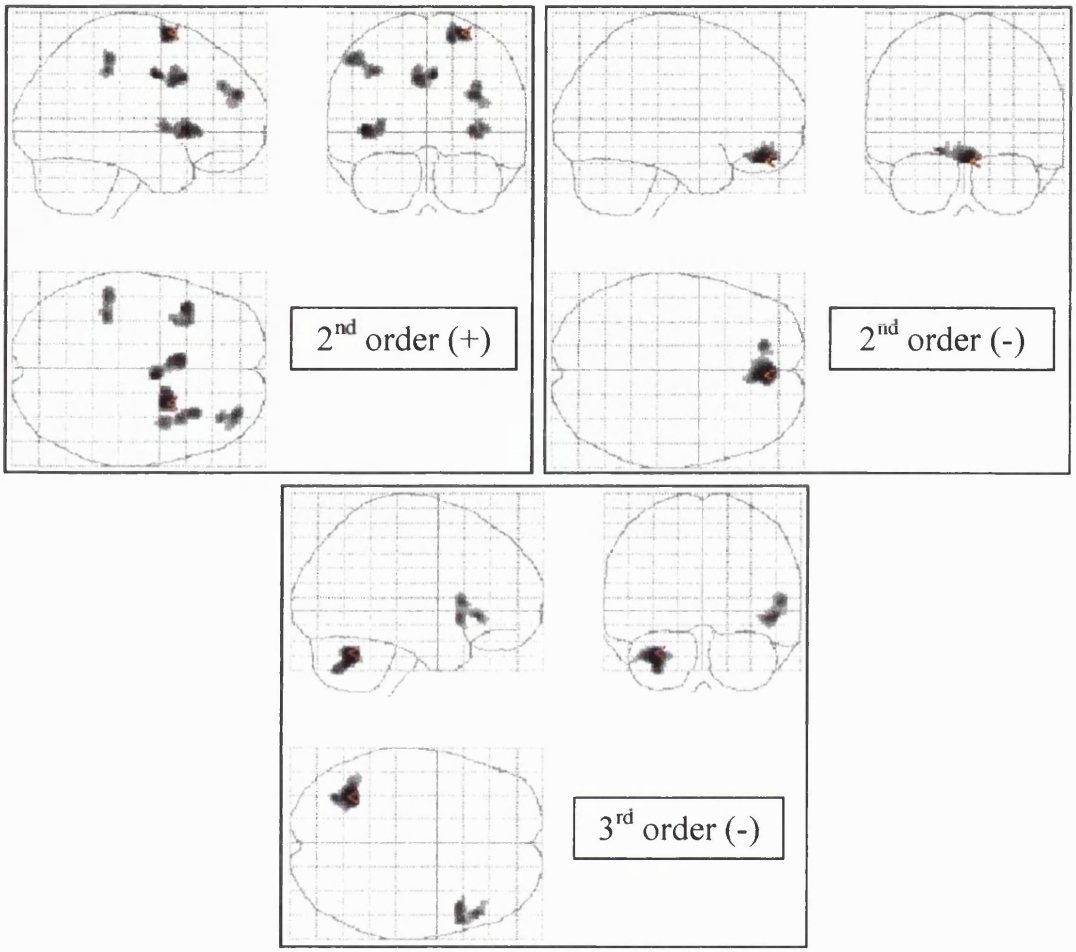


Figure 5.6 SPM{t}s representing regions in which there are significant non-linear relationships between BOLD signal and increasing hand grip force using the dominant hand. The SPM{t}s are shown as maximum intensity projections. The brain is shown from the right, top, and back. All clusters are significant at $p<0.05$, corrected for multiple comparisons across whole brain.

5.3.5 Effects of age

Regions showing a significant relationship in the conjunction of the effects of age^2 AND effects of handgrip are shown in table 5.5 and figure 5.7. When using the dominant hand, positive correlations with age^2 were observed in a number of voxels, particularly in a large cluster in the contralateral (left) hemisphere ranging from y = -36 to y = -4. Peaks within this cluster were situated in postcentral sulcus, inferior central sulcus and precentral gyrus. Further correlations were observed in contralateral caudal cingulate sulcus and ipsilateral superior frontal sulcus, frontal operculum, insula cortex, and intraparietal sulcus, as well as ipsilateral superior cerebellum, cerebellar vermis, ipsilateral ventral posterolateral thalamus and bilateral caudate nuclei. When using the non-dominant hand positive correlations were seen in ipsilateral (left) inferior postcentral gyrus, inferior central sulcus and superior frontal sulcus. Further correlations were observed in contralateral (right) caudal cingulate sulcus, PMd, inferior frontal sulcus (BA 45), cerebellar vermis, and thalamus (mediodorsal nuclei).

Conjunction analysis was also performed between the effect of age^2 AND the effect of rest compared to hand grip. For both dominant and non-dominant hands, significant correlations were seen in ipsilateral primary motor cortex (M1), such that in younger subjects there was more likely to be a deactivation of ipsilateral M1 during hand grip compared to rest (figure 5.8).

There was no significant negative correlation between age^2 and BOLD signal (i.e. increasing signal in younger subjects), and neither were there any regions demonstrating significant correlations between age^2 and linear or non-linear effects of hand grip.

Post hoc regression analysis using age rather than age^2 as the independent variable revealed correlations in the same regions that were less significant.

There was no correlation between MVC and main effects of handgrip for either hand.

There were no differences in activation patterns between male and female subjects.

Table 5.5 *Effects of increasing age*

Region of activation	Talairach coordinates in MNI space x	y	z	Z-score	Correlation coefficient
(a) Conjunction between effects of age^2 and effects of dominant (right) handgrip versus rest					
Left inferior central sulcus	-34	-8	42	6.23	+0.73
Left postcentral gyrus	-24	-34	50	7.13	+0.80
Left precentral gyrus (BA 4/6)	-22	-18	58	5.50	+0.67
Left caudal cingulate sulcus	-8	-6	58	5.32	+0.65
Cerebellar vermis	6	-62	-26	5.41	+0.66
	8	-64	-18	5.30	+0.65
	-6	-68	-34	5.35	+0.65
Right superior cerebellum	22	-46	-24	5.27	+0.64
Right superior frontal sulcus	34	-4	40	5.75	+0.69
Right frontal operculum	42	12	8	5.67	+0.68
Right intraparietal sulcus	34	-58	52	5.62	+0.68
Right insula cortex	34	6	12	5.43	+0.66
Right thalamus (vpl)	12	-24	10	4.97	+0.65
Right caudate	12	4	18	5.15	+0.63
Left caudate	-12	4	16	4.96	+0.64
(b) Conjunction between effects of age^2 and effects of rest versus dominant (right) handgrip					
Right M1	46	-22	56	5.39	+0.66
(c) Conjunction between effects of age^2 and effects of non-dominant (left) handgrip versus rest					
Left inferior central sulcus	-32	-10	42	5.46	+0.66
Left inferior postcentral gyrus	-58	-16	28	5.77	+0.69
Left superior frontal sulcus	-26	-4	44	5.16	+0.64
Cerebellar vermis	8	-72	-36	6.53	+0.75
	10	-64	-14	5.41	+0.65
Right inferior frontal gyrus (BA 45)	50	22	24	5.42	+0.66
Right dorsal premotor cortex	38	-4	56	5.20	+0.63
Right caudal cingulate sulcus	6	-8	42	5.22	+0.64
Right mediodorsal thalamus	8	-22	8	5.22	+0.64
(d) Conjunction between effects of age^2 and effects of rest versus non-dominant (left) handgrip					
Left M1	-28	-30	60	5.44	+0.64

Conjunction of effects of handgrip and effects of age^2. All voxels significant at $p < 0.05$ after correction for multiple comparisons across whole brain. BA = Brodmann area; M1 = primary motor cortex; vpl = ventral posterolateral.

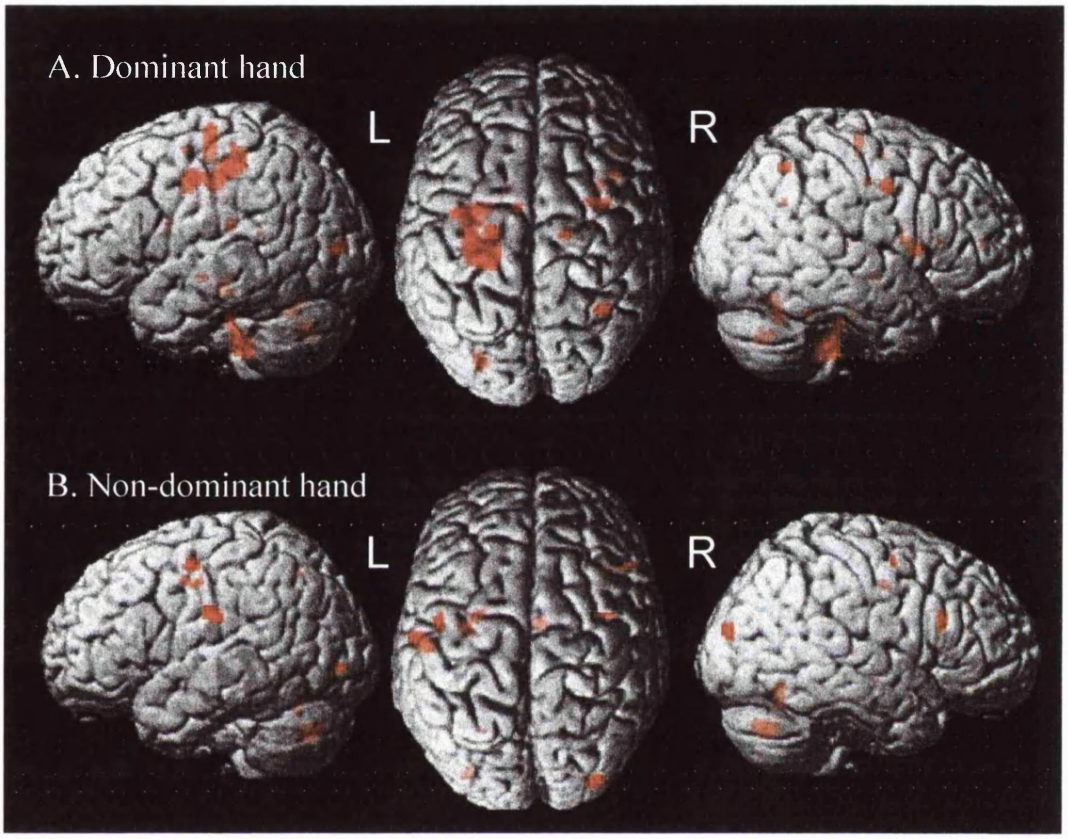


Figure 5.7 SPM{t}s representing the conjunction of main effects of handgrip and effects of age^2 for (A) dominant hand grip, and (B) non-dominant hand grip. Results are surface rendered onto a canonical brain. The brain is shown (from left to right) from the left side, from above (left hemisphere on the left), and from the right. All voxels are significant at $p < 0.05$, corrected for multiple comparisons across whole brain.

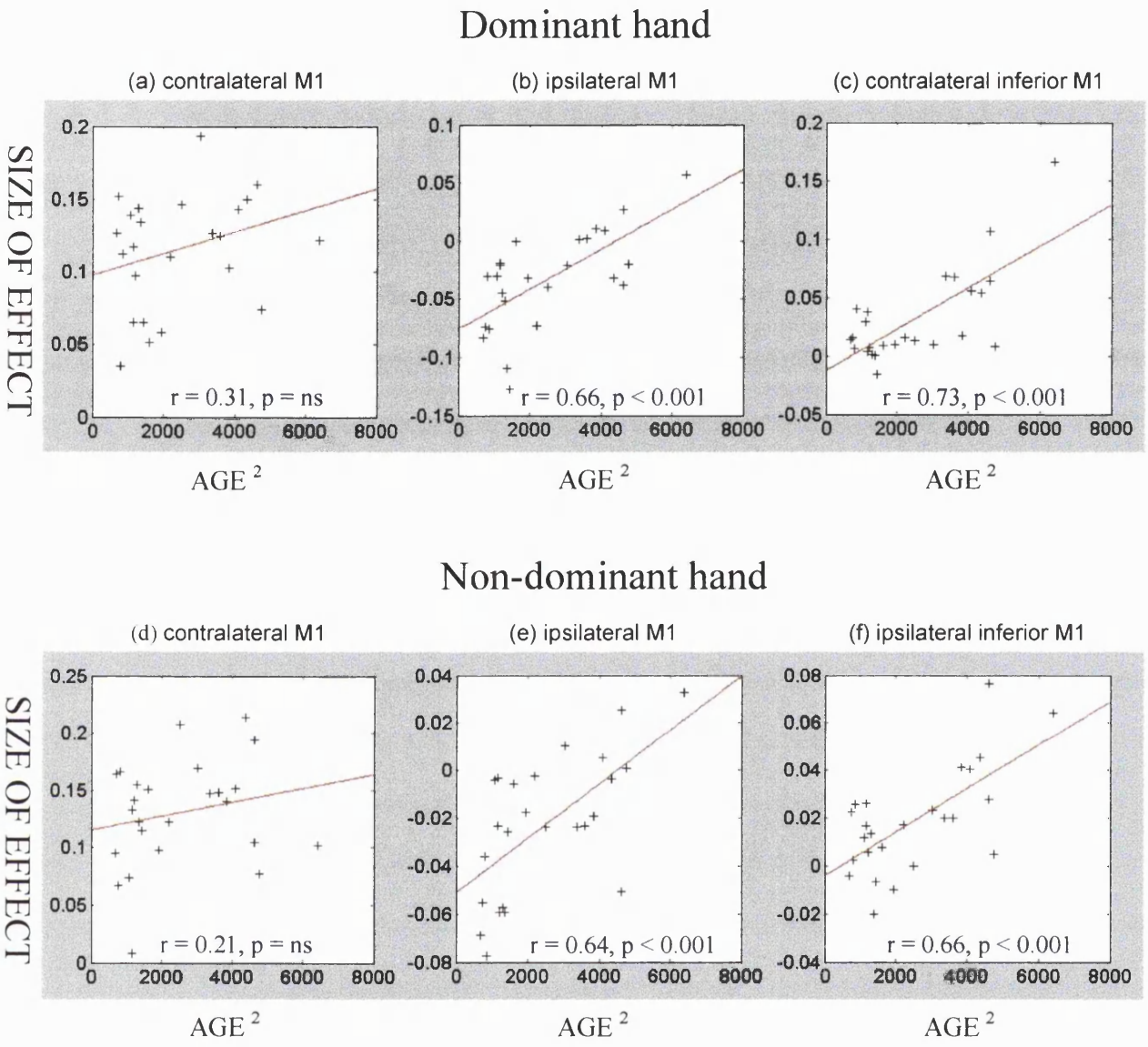


Figure 5.8 Parameter estimates for the main effect of hand grip plotted against age^2 for (a) contralateral M1 (dominant hand, x = -34, y = -30, z = 56), (b) ipsilateral M1 (dominant hand, x = 46, y = -22, z = 56), (c) contralateral (left) inferior M1 (dominant hand, x = -34, y = -8, z = 42) (d) contralateral M1 (non-dominant hand, x = 38, y = -26, z = 52), (e) ipsilateral M1 (non-dominant hand, x = -28, y = -30, z = 60), (f) ipsilateral (left) inferior M1 (non-dominant hand, x = -32, y = -10, z = 42) The correlation coefficient *r* and the corresponding p-value are given for each plot. The parameter estimates are calculated for single peak voxels within each region.

5.4 Discussion

5.4.1 Main effects of handgrip

Previous functional imaging studies involving arm and hand movements have demonstrated activation in contralateral primary sensorimotor cortex, ipsilateral anterior cerebellum, superior parietal cortex and ventrolateral thalamus (Grafton *et al.*, 1991, 1992; Colebatch *et al.*, 1991). The results of this experiment have shown that isometric dynamic handgrip with visual feedback activates a more extensive network of cortical and subcortical regions, all known to be part of the motor system (Fink *et al.*, 1997). Of particular interest is the finding of bilateral activation in the rostral part of PMv (BA 44) and intraparietal sulcus when either hand is used. There is strong evidence to support the notion that BA 44 in humans is the functional homologue of area F5 in the macaque (von Bonin and Bailey, 1947), both from comparative architectonic analysis (Petrides and Pandya, 1994), and functional imaging studies (Binkofski *et al.*, 2000; Rizzolatti *et al.*, 1996). In addition, intraparietal sulcus in humans is likely to be the functional homologue of anterior intraparietal area (AIP) in the macaque (Faillenot *et al.*, 1997; Binkofski *et al.*, 1998). Area F5 is directly connected to primary motor cortex, and receives inputs from the secondary somatosensory area (SII) and posterior parietal cortex, particularly AIP (Muakkassa and Strick, 1979; Ghosh and Gattera, 1995; Luppino *et al.*, 1999). On the basis of intracortical microstimulation studies, both F5 and AIP have been implicated in a network of cortical regions associated with grasping (Rizzolatti *et al.*, 1981, 1988; Kurata and Tanji 1986; Taira *et al.*, 1990; Hepp-Reymond *et al.*, 1994). The notion of a 'grasping circuit' involving these structures was described by Jeannerod (1995), and the human equivalent is

thought to involve BA 44 together with intraparietal sulcus. However, until recently such a network had not been demonstrated in humans (Grafton *et al.*, 1996; Rizzolatti *et al.*, 1996). Studies placing the emphasis on the exploratory (Binkofski *et al.*, 1999) or precision (Ehrsson *et al.*, 2000, 2001) nature of grip succeeded in demonstrating activation of BA 44 and intraparietal sulcus. The hand grip task used in this experiment also activated BA 44 and intraparietal sulcus/supramarginal gyrus bilaterally with either hand. Such findings lend further support to the notion of a human grasping network involving these regions. Given the diversity of tasks that activate this network (blinded object manipulation, finger thumb precision grip with tactile feedback, and now hand grip with visual feedback), I suggest that it is the continued monitoring of hand performance that is important, rather than the type of hand task. Lesion studies in humans have suggested that left BA 44 is involved in 'on-line' control of visually guided movements (Jackson and Husain, 1996), whereas right BA 44 may have a role in directing attention as it has been associated with unilateral visual neglect in humans (Husain and Kennard, 1996). I am not able to define this differential functionality any further on the basis of my results.

5.4.2 Correlation between BOLD signal and handgrip force

Using single cell recording in macaque monkeys (Ashe, 1997), the correlation between firing rates of cortical neurons and force of both isometric and dynamic grip tasks has been demonstrated to be mainly monotonic or linear. Linear relationships have been observed primarily for neurons in M1 (Evarts, 1968; Evarts *et al.*, 1983; Hepp-Reymond *et al.*, 1978; Smith *et al.*, 1975; Wannier *et al.*,

1991; Georgopoulos *et al.*, 1992), and also somatosensory cortex (Wannier *et al.*, 1991), premotor cortex (Hepp-Reymond *et al.*, 1994), SMA (Smith *et al.*, 1975), and thalamus (Anner-Baratti *et al.*, 1986). Functional imaging techniques allow the activity in larger populations of neurons to be studied simultaneously across all brain regions, which allows inference about activity relationships between one population of neurons and another. However, local differences in behaviour of neurons will be masked. Despite this limitation, Dettmers *et al.*, (1995) found linear increases in regional cerebral blood flow (rCBF) with increasing force of finger key presses in a number of regions, particularly contralateral M1, primary sensory cortex, caudal cingulate sulcus, posterior SMA (SMA proper), cerebellar vermis and ipsilateral thalamus. I was able to replicate these findings, with the exception of SMA. Using a dynamic hand grip task, Thickbroom *et al.*, (1999) demonstrated an increase in extent but not size of BOLD signal in contralateral motor cortex in relation to increasing force, and were unable to demonstrate any relationship with force using a static grip task (Thickbroom *et al.*, 1998). Compared to the dynamic grip task of Thickbroom *et al.* (1999) my task was performed across a much wider range of forces (10-60% MVC) making it more sensitive to detection of such changes.

I found significant non-linear correlations of brain activity with increasing force in multiple regions of the brain in individual subjects. There was however significant individual variability. The absence of any consistent non-linear correlates across the group when using the non-dominant hand suggests that the variability is more marked using this hand. Furthermore, I report dominant hand group effects at the cluster level, with no significant single voxels at the chosen threshold ($p < 0.05$,

corrected for multiple comparisons for whole brain). Non-linear changes in firing rates of single cortical cells in precentral gyrus of the macaque monkey have been reported (Cheney and Fetz, 1980; Evarts *et al.*, 1983), particularly in higher or lower force ranges. These non-linear responses have been interpreted as either recruitment or saturation effects of different populations of neurons within functionally similar cortical regions. This hypothesis is supported by the observation that the BOLD signal in voxels within the same cortical region behaved in very different fashion in relation to increasing force, e.g. voxels within right insula cortex exhibited both positive second order effects and negative third order effects (group analysis - table 5.4). Recent evidence has suggested an alternative explanation. Hepp-Reymond *et al.* (1999) investigated context specific force encoding in a number of premotor regions and primary motor cortex of the macaque monkey. A number of neurons were found in all sites in which the rate of change of firing was dependent on context, i.e. context may change the gain control of the motor system. For example, it is possible that increased attention to accuracy is required at both low and high forces. This might explain the positive second order effect seen in regions such as intraparietal sulcus, dorsolateral prefrontal cortex, insula cortex and cingulate sulcus. In my experimental design, I did not include explicit contextual or strategic changes, but clearly further work is required to establish whether this phenomenon occurs in the human motor system.

5.4.3 Effects of age

Two previous studies made categorical comparisons between an older and a younger group using the dominant hand to demonstrate relative overactivity in a

number of motor related regions and one study failed to show a difference using the non-dominant hand (Calautti *et al.*, 2001c; Mattay *et al.*, 2002). Such categorical comparisons may miss more subtle age related changes so I chose to perform a correlation analysis. Linear age related changes in performance have been demonstrated using motor tasks such as repetitive finger tapping (Shimoyama *et al.*, 1990), but non-linear effects are seen in more demanding timed tasks, choice reaction times and visually guided hand movements (Kauranen *et al.*, 1996; Houx *et al.*, 1993; Smith *et al.*, 1999). I asked my subjects to be accurate in exerting the target force during each hand grip, increasing the demands of the task. My *a priori* hypothesis, therefore, was that I would see non-linear effects. The notion that this task represents a precise and relatively demanding motor task compared to finger tapping, for example, is supported by the similarities seen between the regions of activation I found for hand grip and those seen in other studies involving precision (Ehrsson *et al.*, 2000, 2001). The subsequent discussion refers only to the correlation analysis performed using age^2. Activity in the part of contralateral M1activated by hand grip across the group did not show a correlation with age^2. However, activity in ipsilateral hand area of M1 was reduced during hand grip compared to rest in younger subjects, but the degree of relative 'deactivation' was lesser in older subjects (figure 5.8). This finding suggests why significant deactivations reported with ipsilateral hand movement in other studies (Allison *et al.*, 2000) were not seen when averaging across the group in my study. The mechanism of ipsilateral deactivation of M1 is thought to be via transcallosal inhibition. This process may be reduced in older subjects. In support of this idea paired pulse transcranial magnetic stimulation (TMS) has

demonstrated reduced intracortical inhibition in motor cortex of older subjects (Peinemann *et al.*, 2001). It is possible that ageing also leads to impaired transcallosal inhibition of ipsilateral M1.

Increased activation with age was seen in a number of left sided areas. In particular a positive correlation with age^2 was seen in a region deep within left central sulcus corresponding to the putative area 4p (Geyer *et al.*, 1996), whichever hand was used. Area 4p has been shown to be increasingly activated with increasing attention to motor performance (Binkofski *et al.*, 2002), which is consistent with the notion of increased utilisation of neural resources in older subjects in order to maintain performance levels (Mattay *et al.*, 2002). This finding is also interesting in view of studies that have suggested that the left hemisphere, in right handed subjects, is dominant for controlling most cognitive aspects of movement with either hand (Haaland and Harrington, 1996). It has previously been assumed that this is related to a dominance of left sided association areas, such as prefrontal and parietal cortex (Haaland and Harrington, 1996). However, the absence of age related changes in these association areas in my data supports the idea that the left sensorimotor cortex itself plays a role in this hemispheric difference.

Age related increased activations were also seen in regions close to the putative 'grasping circuit'. Older subjects were more likely to recruit right frontal operculum and intraparietal sulcus with dominant hand use, and right inferior frontal gyrus with non-dominant hand use. These frontal regions are both very close to BA 44, with a likelihood of being within BA 44 of 5-25% according to the probability map of Tomaiuolo *et al.* (1999). If this excess activation is in

compensation for increasing cognitive demands of the task in older subjects, it again provides evidence to support a functional differentiation between the networks in dominant and non-dominant hemispheres.

Other age related increases in activation were seen in caudal cingulate sulcus (contralateral when either hand was used) and dorsal premotor cortex/superior frontal sulcus (BA 6/8) regions (bilateral for non-dominant hand and ipsilateral for dominant hand). Caudal regions of the cingulate sulcus are activated in many simple motor tasks, and preferentially during sequential movement (Dieber *et al.*, 1999). Movement complexity has not been shown to alter cingulate activation (Shibasaki *et al.*, 1993). The dorsal premotor (PMd) regions activated increasingly in my older subjects are situated rostrally within the premotor region. It has been suggested that rostral premotor cortex (or pre-PMd) is more sensitive to sensory cues involved during movement (Picard and Strick, 2001). This would be in keeping with the notion that older subjects might find the task more demanding of higher level cognitive processes.

Although I have demonstrated an increase in activations in older subjects, it is not clear whether this is due to a change in threshold for activation, or a change in gain setting. I was interested therefore to see if there were any age related differences in the relationship between force exerted and BOLD signal across the group. I observed a number of brain regions in which older subjects were more likely to show a linear increase in BOLD signal in relationship to increasing force, including contralateral primary motor cortex, right BA 44/45, bilateral intraparietal sulcus/supramarginal gyrus, and bilateral secondary somatosensory area (SII) ($p < 0.001$, uncorrected for multiple comparisons). None of these

regions was significant at the threshold used in this experiment, but it is interesting to speculate that the trend represents an increase in gain setting in older subjects. A task with greater cognitive demand may be better suited to demonstrate this difference.

Changes downstream from the cortical and subcortical motor structures also have an influence on performance. Decline in muscle mass and strength is seen in older human subjects (Buckwalter *et al.*, 1993; Ranganathan *et al.*, 2001), and loss of anterior horn cells has been reported in older animals (Machado-Sallas *et al.*, 1977). Both might impair the transformation of descending corticospinal impulses into the generation of force, and lead to an increased effort in maintaining task performance. However, I asked subjects to perform at a proportion of their own maximal MVC. Effort is therefore largely controlled for in my experimental task making these peripheral changes a less likely explanation for the results.

5.4.4 Conclusions

Isometric dynamic hand grip is a robust activator of a widespread network of cortical and subcortical regions in the human brain. By incorporating 'on-line' visual feedback, I have demonstrated activation in the 'grasping circuit' involving bilateral rostral PMv (BA 44) and intraparietal sulcus, previously seen only in tasks involving pinch grip and blinded object manipulation. Within this motor network, I have demonstrated significant changes in the way the motor system is activated with increasing age. One explanation is that neurodegenerative and neurochemical changes occurring as part of the normal ageing process result in less efficient integration of visuospatial and sensorimotor processing. Thus for an

older subject to perform the task to the same level as a younger subject requires greater computational effort, which is reflected at the systems level as increased activation in key regions. There are clear parallels with changes seen in studies in which the complexity of motor task is increased (Catalan *et al.*, 1998), or in which naïve subjects learn a new motor task (Toni *et al.*, 1998; Karni *et al.*, 1995). In both cases alterations in task difficulty are reflected in adaptive changes in a motor network. Taken together with my findings, these results reflect the adaptable and plastic nature of the networks that subserve motor function in response to increasing demands relating to task complexity or neuronal loss. An extreme example of this is seen in patients suffering motor deficit as a result of stroke who appear to rely on this adaptability as the substrate for some part of their functional recovery (Chollet *et al.*, 1991; Weiller *et al.*, 1993). I will investigate this phenomenon in the subsequent 3 chapters.

However, the capacity for plastic change may be finite. After injury induced reorganisation of the brain, the capacity for subsequent adaptive change is reduced (Kolb *et al.*, 1998). It is possible that the adaptive changes I have observed in older brains may in turn limit the capacity for further reorganisation after injury. This clearly has implications for what can be expected from therapeutic techniques designed to promote cerebral reorganisation after stroke in older subjects. A greater understanding of age related changes in the functional reorganisation of the brain will be crucial to unraveling the relationship between normal ageing and pathological process.

Chapter 6

NEURAL CORRELATES OF OUTCOME AFTER STROKE:

A CROSS-SECTIONAL FMRI STUDY

6.1 Introduction

Functional imaging has been used to investigate cerebral reorganisation after focal brain damage in humans for a number of years. Several contributions have been made towards our understanding of the way the brain responds to injury, as discussed in chapter 2.

However, to date these studies do not provide a clear link between observed changes in brain activation patterns and functional outcome. An understanding of this relationship is crucial if functional imaging is to play an important role in establishing the neurobiological basis of functional recovery after focal brain damage.

Having characterised the functional anatomy of dynamic hand grip I was able to use this task to investigate brain activation patterns in a variety of chronic stroke patients. By studying a number of previously hemiparetic patients with variable functional outcomes, I was able to address the null hypothesis that there is no relationship between outcome after stroke and the size, extent or location of activation on a voxel by voxel basis.

6.2 Materials and methods

6.2.1 Subjects

Patients were recruited from the outpatient and inpatient services at the National Hospital for Neurology and Neurosurgery, Queen Square, London. All patients had suffered from first-ever stroke not less than three months previously, resulting in weakness of at least wrist and finger extensors and hand interossei (to ≤ 4+ on the Medical Research Council (MRC) scale), for at least 48 hours after onset of symptoms. Exclusion criteria consisted of (1) carotid artery occlusion or stenosis ≥ 70%; (2) language or cognitive deficits sufficient to impair co-operation in the study; (3) inability to perform the motor task due to complete paralysis of hand grip. All patients received post stroke rehabilitation therapy appropriate to their clinical needs. The age-matched control group is described in chapter 5.

All patients and control subjects were right handed according to the Edinburgh handedness scale (Oldfield, 1971). Full written consent was obtained from all subjects in accordance to the Declaration of Helsinki. The study was approved by the Joint Ethics Committee of the Institute of Neurology and National Hospital for Neurology and Neurosurgery, UCL Hospitals NHS Trust, London.

6.2.2. Behavioural evaluation

Patients were evaluated using a battery of outcome measures, designed to assess different aspects of functional outcome after stroke. These have been described in detail in chapter 4. All patients were scored on each outcome measure on the same day as magnetic resonance imaging (MRI). In comparing these scores, the Rankin disability scale and Orpington Prognostic Scale were converted such that increasing scores reflected improvement, by subtracting the measured score from the maximum score possible for that scale. Thus for each outcome measure there were 20 scores, one for each patient. Each set of outcome measures was normalised (giving unit variance and zero mean), creating nine sets of outcome measures, or vectors (one each for Barthel, Rankin, OPS etc.). In order to obtain one representative vector of outcome, I performed a principal component analysis on the whole data set, as described in chapter 4. The first principal component (a vector of 20 values, one value for each patient) was taken as the vector representing outcome across the patient group. The size of brain activation in each voxel across subjects was correlated with this vector.

6.2.3 Motor paradigm

The isometric dynamic hand grip task is described in chapter 5. Patients performed this task using the impaired hand. Control subjects performed the task with dominant and non-dominant hands in separate sessions and in a randomised counterbalanced order to provide control data for patients with dominant or non-dominant affected hands.

Prior to scanning, but whilst lying in the scanner, patients were asked to grip the manipulandum with maximum force to generate a maximum voluntary contraction (MVC). Measurement of MVC was made with the patient in the same position as during the scanning because changes in posture can affect motor performance in stroke patients. Target forces during scanning were set at 10%, 20%, 40% and 60% of MVC for each patient, and were indicated by a horizontal bar on the screen. The required rate of hand grip was indicated visually by a cross displayed at the bottom of the screen for 0.3 seconds at a rate of 40% of maximum rate. Thus, although the absolute parameters of the task varied between patients, the effort exerted during the task was equalised, as discussed in chapter 4.

All patients performed the motor task outside the scanner to look for associated movements or mirror movements. To aid this assessment, patients held two identical hand grip manipulanda, one in each hand, during repetitive hand grip with the affected hand. Simultaneous recordings were made from both hands to enable detection of true mirror movements (Nelles *et al.*, 1998). In addition surface electromyogram (EMG) electrodes were positioned on biceps, triceps, and latissimus dorsi bilaterally, to detect more proximal muscle activation.

6.2.4 Data acquisition

A Siemens VISION system (Siemens, Erlangen, Germany), operating at 2 T, was used to acquire both T_1-weighted anatomical images (1 x 1 x 1.5 mm voxels) and T_2*-weighted transverse echo-planar (EPI) MR images (64 x 64 3 x 3 mm^2 pixels, T_E = 40ms) with blood oxygenation level dependent (BOLD) contrast. Each echoplanar image comprised forty eight 1.8 mm thick contiguous axial slices

taken every 3mm, positioned to cover the whole cerebrum. A total of 270 volumes were acquired continuously during each session, with an effective repetition time (T_R) of 3.649 seconds per volume. The first six volumes were discarded to allow for T_1 equilibration effects.

6.2.5 Image analysis

The acquired volumes were realigned and unwarped as described in Chapter 5. The standard normalisation process may result in incorrect normalisation (and therefore incorrect localisation of activations) in brains with abnormal structure. In order to mitigate this in the stroke patients, a mask of the lesion was created using MRIcro software (MRIcro, Nottingham University, http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html). This mask was incorporated into the normalisation step for all patients (Brett *et al.*, 2001). All normalised images were then smoothed with an isotropic 8 mm full-width half-maximum Gaussian kernel to account for intersubject differences and allow valid statistical inference according to Gaussian random field theory (Friston *et al.*, 1995b). The time series in each voxel were high pass filtered at 1/100 Hz to remove low frequency confounds and scaled to a grand mean of 100 over voxels and scans within each session.

Statistical analysis was performed in two stages, as in chapter 5. Thus a fixed effects model was used for all patients. All handgrips were defined as a single event type, and modelled as delta functions, together with orthogonalised polynomial expansions of the peak grip force for each hand grip/event. The resulting covariates were convolved with a canonical synthetic haemodynamic

response function, and were used in a general linear model (Friston *et al.*, 1995a) together with a single covariate representing the mean (constant) term over scans. The parameter estimates for each covariate resulting from the least mean squares fit of the model to the data were calculated, and statistical parametric maps of the t-statistic (SPM{t}) resulting from linear contrasts of each covariate (Friston *et al.*, 1995a) were generated and stored as separate images for each subject.

The first null hypothesis states that there is no difference in the task related activation pattern for an individual patient compared to the normal population. The data for the second stage of analysis comprised two samples; (1) the pooled parameter estimates for the covariate representing the main effects of hand grip across control subjects (during the use of dominant or non-dominant hand as appropriate for each patient); (2) the parameter estimate for the same covariate in a single patient. Thus, contrast images for each subject were entered into two sample t-tests (control group compared to single patient) in a random-effects analysis (Friston *et al.*, 1999). The SPM{t}s were thresholded at $p < 0.05$, corrected for multiple comparisons across whole brain.

The second null hypothesis states that there is no correlation between the voxel-wise task related change in BOLD signal and the outcome across subjects. This question was addressed in two ways. Firstly, using a fixed effects model, to make inferences about whether such a correlation exists within subgroups of patients with the same cerebral infarct location. Secondly, using a random effects model incorporating all patients in order to extend the inference into the population from which the sample was drawn. For all group correlation analyses, the brains of

patients with right hand weakness were flipped about the mid-sagittal line, such that all subjects were considered to have right-sided infarcts.

In the subgroup analyses, multi-subject fixed effects models were employed, using the same covariates as described for single subject analysis. I examined for voxels in which a linear correlation between outcome scores and the parameter estimates for the covariates representing the main effects of hand grip across subjects existed. The specific contrast across the appropriate covariates was weighted according to the mean corrected outcome scores. The resulting SPM{t}s were thresholded at $p < 0.05$, corrected for multiple comparisons across whole brain.

In the random effects model, I performed a linear regression analysis within SPM99, in which the variables consisted of the contrast images representing the main effect of hand grip for each patient, and a measure of outcome for each patient (mean corrected across the group). For significant voxels, the correlation coefficient for the plot of parameter estimate against outcome for each subject, together with the corresponding *p*-value, were calculated.

All SPM{t}s were transformed to the unit normal Z-distribution to create a statistical parametric map (SPM{Z}).

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).

6.3 Results

6.3.1 Clinical data

Twenty stroke patients were recruited (range 28 to 72 years). The mean age of the patient group was 53.2 ± 14.5 years, compared to 47.6 ± 15.8 years in the control group. Patient characteristics are listed in table 6.1. Fourteen patients had experienced left hemiparesis, and 6 right hemiparesis. The site of cerebral infarction was determined from the T_1-weighted structural MRI. Eight patients had infarcts involving the internal capsule or corona radiata (IC), four patients had pontine infarcts (pons), and six patients had infarcts in a striatocapsular distribution with extension to the insula cortex (MCA) (figure 6.1). In addition, one patient had suffered from a thalamic haemorrhage, and one from haemorrhagic infarction of the posterior middle cerebral artery territory. No patients had lesions involving the hand representation of M1.

Outcome at the time of scanning was variable as measured by the selected outcome scores (table 6.2). The first principal component of the data set comprising scores for each outcome measure across patients accounted for 81.2% of the variance and was taken as the vector of overall outcome across the group (figure 6.2). The proportion of each individual outcome score to the first principal component is given in table 6.2.

6.3.2 Behavioural results

All controls and patients were able to perform the task adequately. No subject displayed mirror movements at bedside observation. When performing the motor

Figure 6.1 Axial structural T_1-weighted MRI scans at the level of maximum infarct volume for each patient performed at the time of the functional MRI. The front of the brain is upwards, right side of the brain displayed on the left.

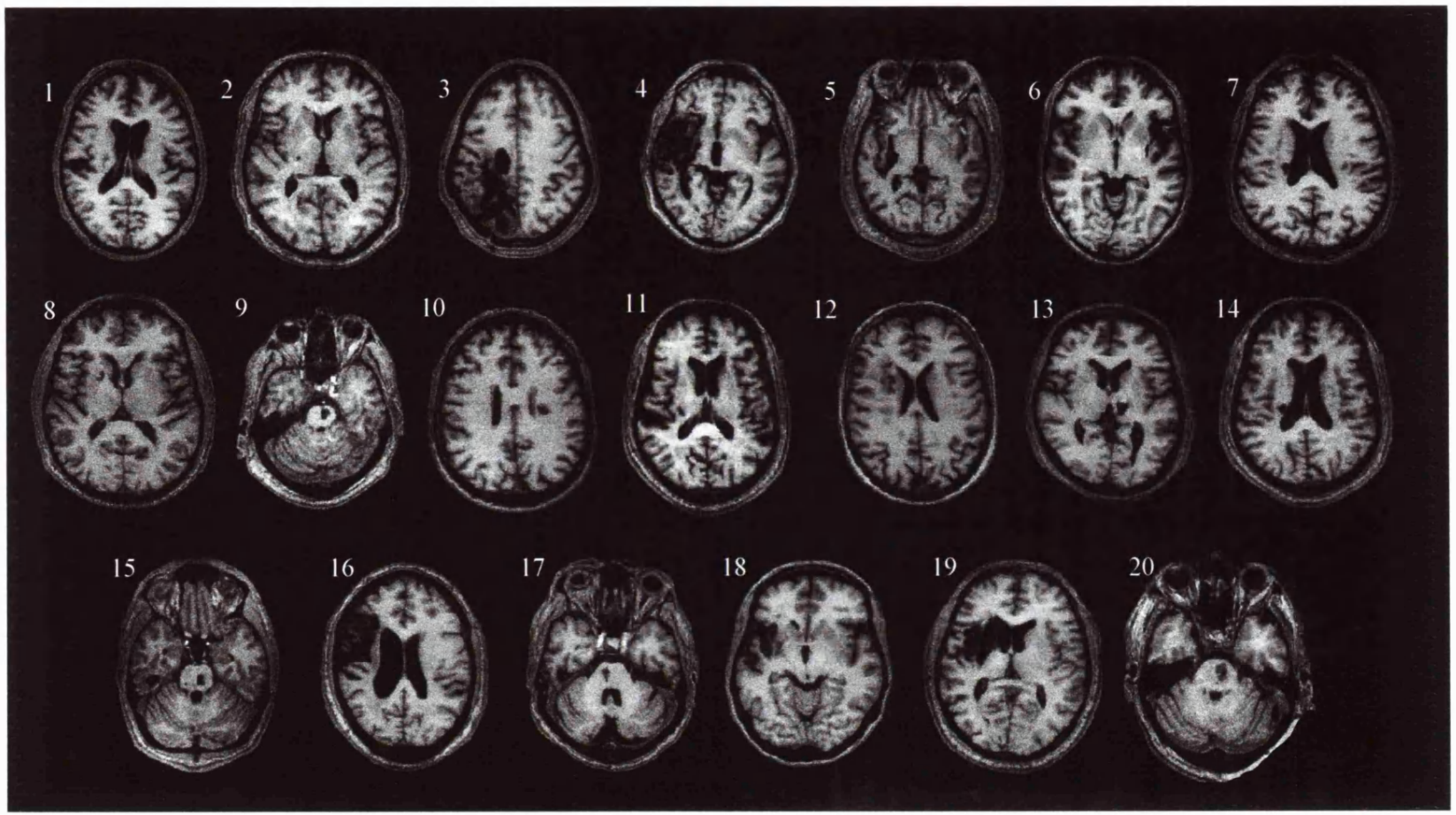


Table 6.1 *Patient characteristics*

Patient	age	sex	affected hand	Site of lesion	Time to scan	Initial Severity*	PMH	Medication
1	50	M	L	R IC	13 weeks	3	NIDDM	aspirin, simvastatin, gliclazide, metformin
2	56	M	L	R IC	29 weeks	4+	nil	Aspirin
3	39	M	L	R posterior MCA haemorrhagic infarct	6 years	0	epilepsy	sodium valproate
4	69	M	L	R MCA	3 years	0	hyperthyroidism	aspirin, thyroxine
5	49	M	L	R MCA	18 weeks	0	hypertension	aspirin, losartan, bendrofluazide
6	29	F	R	L MCA	13 weeks	2 (1)	nil	Aspirin
7	58	M	L	R IC	25 weeks	2	hypertension	aspirin, bendrofluazide
8	63	M	L	R IC	21 weeks	4+ (4)	hypertension	aspirin, atenolol, amlodipine, simvastatin
9	71	M	R	L pons	28 weeks	2	IHD, COPD	aspirin, glyceryl trinitrate spray
10	52	M	R	L IC	27 weeks	4+	hypertension	aspirin, atenolol, ramipril
11	72	M	L	L IC	3 years	1	mild asthma	Aspirin
12	72	F	L	R IC	15 weeks	1	hypertension,	aspirin, bendrofluazide, atorvastatin
13	50	M	R	L thalamic haemorrhage	27 weeks	0	hypertension, gout	atenolol, lisinopril, allopurinol, omeprazole
14	60	M	L	R IC	14 weeks	4+	hypertension	aspirin, bendrofluazide
15	29	M	R	L pons	36 weeks	0	nil	aspirin, fluoxetine
16	46	M	L	R MCA	27 weeks	0	hypertension	aspirin, amlodipine, atenolol, atorvastatin
17	61	M	L	R pons	14 weeks	2 (0)	hypertension	aspirin, atenolol
18	28	F	L	R MCA	4 years	0	nil	Aspirin
19	38	M	L	R MCA	35 weeks	0	nil	Aspirin
20	71	M	R	L pons	20 weeks	0	hypertension	aspirin, atenolol

* Initial severity refers to the Medical Research Council grade for wrist extension (and finger extension in brackets if different) as recorded in the medical records at the time of stroke.

M = male, F = female, R = right, L = left, IC = internal capsule, MCA = middle cerebral artery, NIDDM = non-insulin dependent diabetes mellitus, IHD = ischaemic heart disease, COPD = chronic obstructive

Table 6.2 *Patient outcome scores*

Patient	Barthel	Rankin	OPSS	ARAT	MI (UL)	MI (LL)	10m walk	NHPT	Grip strength	Ashworth scale	Sensory loss	Mirror movements	Associated movements	Overall Outcome Score
	0 - 20	5 - 0	1.6 - 6.8	0 - 57	0 - 100	0 - 100	m/s	% contralateral side		5 - 0				
	20	2	2	51	93	92	0.6	66.7	75.2	0	no	no	no	0.06
2	20	1	1.6	57	100	100	1.4	75.4	102.2	0	face, hand	no	no	0.2
3	20	3	2.4	14	61	59	0.8	0	40.7	2	UL,LL	no	WF	-0.22
4	18	3	2.4	24	70	100	0.9	0	58.2	1	UL,LL	no	no	-0.12
5	10	4	3.2	30	58	59	0	3.7	35.5	0	UL,LL	no	no	-0.36
6	20	1	2	57	100	100	1.6	78.4	90.2	1	no	no	no	0.18
7	19	2	2.8	38	78	62	0.4	64.1	77.1	3	no	no	WF,EF	-0.08
8	20	1	1.6	57	100	100	1.5	107.3	91.2	0	no	no	no	0.21
9	20	1	2	57	100	100	1.1	88.4	98.2	1	no	no	no	0.18
10	20	1	1.6	57	100	100	2.3	102.8	103	0	no	no	no	0.26
11	10	3	2	10	58	59	0.5	0	54.9	2	no	no	WF	-0.28
12	20	1	1.6	57	92	100	1.1	55.9	57.8	0	hand	no	no	0.11
13	20	2	1.6	57	92	100	1.3	86.7	96.7	0	no	no	no	0.16
14	20	2	1.6	57	92	100	1	75	91,5	0	hand	no	no	0.13
15	20	1	1.6	57	100	100	1.4	80.6	98.3	0	no	no	no	0.2
16	15	4	3.2	14	58	59	0	11.7	17.8	1	no	no	WF	-0.37
17	16	4	2.8	49	77	92	0	86.2	54.3	3	no	no	no	-0.11
18	20	1	2	56	92	100	1.2	29.1	85.6	2	no	no	no	0.11
19	20	1	1.6	57	100	100	1.5	68.1	106.9	0	no	no	no	0.2
20	9	4	4	15	48	62	0	0	26.8	1	UL,LL	no	WF,EF	-0.45
Contribution of score to overall Outcome score	10.46%	11.40%	10.89%	11.36%	12.19%	10.95%	10.79%	10.38%	11.58%					

Minimum to maximum scores (or units) expressed under each outcome score.

OPSS = Orpington Prognostic Stroke Scale, ARAT = Action Research Arm Test, MI = Motricity Index, NHPT = nine hole peg test, WF = wrist flexion, EF = elbow flexion, UL = upper limb, LL = lower limb.

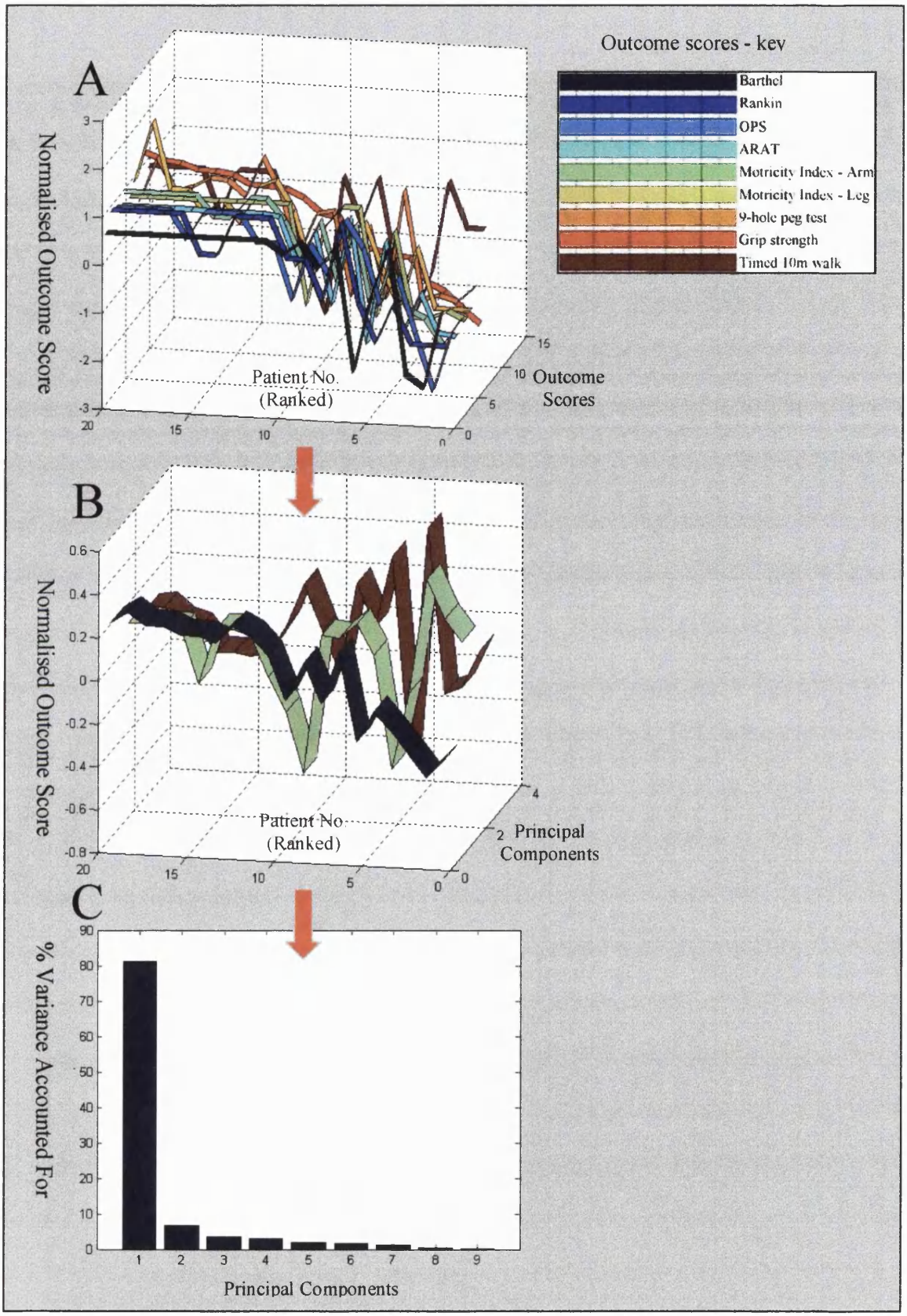


Figure 6.2 (A) Normalised outcome scores for all 20 patients (ranked by hand grip force – red ribbon). (B) First, second and third principal components of outcome data. (C) % Variance of outcome data set explained by each of the principal components.

paradigm outside the scanner there was no evidence of mirror movements or surface EMG activity in biceps, triceps or latissimus dorsi muscles on the side opposite the previously paretic hand. However, a number of patients did exhibit synergistic flexion of wrist and elbow, as well as shoulder adduction during rehearsal of the motor paradigm (table 6.2). A 100 mm visual analogue scale (VAS) (where 0 = 'no effort' and 100 = 'maximum effort') used after scanning, suggested no significant differences existed in the perceived effortfulness of the task across subjects (range 10 to 33, mean 22.2). There was no correlation between the rating for effort and the overall outcome score for each patient (r^2= 0.08, p = ns).

6.3.3 Comparison of single patients to control group

In the comparison of the main effects of hand grip for single stroke patients compared to the control group, the task related activation patterns of five patients were indistinguishable from the control group (at a threshold of $p < 0.05$, corrected for multiple comparisons across the whole brain). These patients were more likely to have made a good recovery. In general, patients with poorer outcome were more likely to activate a number of brain regions over and above those activated in the control group performing the same task (table 6.3, figure 6.3). These regions were often bilaterally distributed, involving sensorimotor, premotor, posterior parietal, prefrontal and insula cortices, supplementary motor area (SMA), cingulate motor areas (CMA), and cerebellum. Increased putaminal activation was seen in only four patients, and thalamic overactivation in one.

	poorer outcome ← Patients ranked by outcome score → better outcome																			
Region	20	16	5	11	3	17	4	7	1	18	12	14	13	9	6	15	2	19	8	10
SMC - IL	+	+	+		+		+													
SMC - CL	+	+	+	+	+		+													
PMd - IL	+				+		+	+									+			
PMd - CL	+	+	+	+	+	+	+	+												
PMv - IL						+	+													
PMv - CL	+	+		+			+	+	+											
CMA - IL		+	+	+	+	+	+													
CMA - CL		+	+				+													
SMA - IL	+	+	+	+			+													
SMA - CL	+	+	+	+				+												
PPC - IL	+	+		+		+	+		+											
PPC - CL	+	+	+	+	+	+	+		+								+			
PFC - IL	+	+	+		+	+	+													
PFC - CL	+	+		+	+	+	+	+									+			
Insula Cortex - IL			+	+	+	+														
Insula Cortex - CL		+		+			+													
Cerebellum - IL	+	+	+	+	+		+	+			+		+							
Cerebellum - CL	+	+	+	+	+		+	+						+			+			
Cerebellar vermis		+	+		+	+	+						+	+						
Putamen - IL		+			+	+														+
Putamen - CL		+					+													
Thalamus - IL		+																		
Thalamus - CL		+															+			

Table 6.3 illustrates the finding that patients with poorer outcome activated more brain regions than patients with better outcome. Each column shows the areas of increased task related activation for an individual patient compared to controls. Patients (represented by patient number - see table 6.1) have been ranked by outcome.

+ = Increased activation present in patient compared to control group (voxels significant at $p < 0.05$, corrected for multiple comparisons across whole brain volume)

SMC = sensorimotor cortex, PMd = dorsolateral premotor cortex, PMv = ventrolateral premotor cortex, CMA = cingulate motor area, SMA = supplementary motor area, PPC = posterior parietal cortex, PFC = prefrontal cortex, IL = ipsilesional, CL = contralesional.

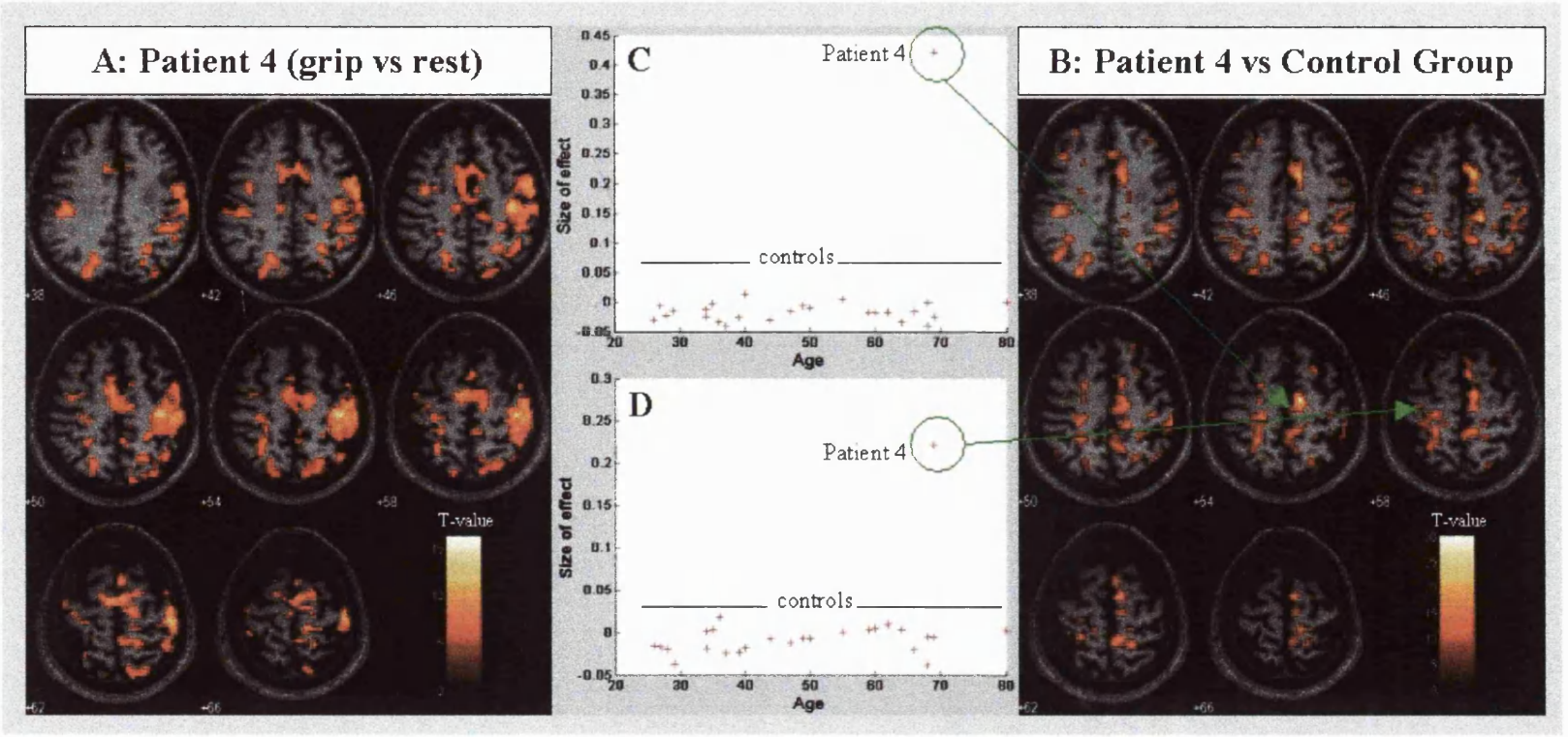


Figure 6.3 Results for a single stroke patient (patient 4) with only moderate outcome. (A) The main effects of left (affected) hand grip compared to rest and displayed on patients own structural *T*1-weighted anatomical image in axial section. The front of the brain is upwards, right side of the brain displayed on the right. (B) The comparison of the main effects of left hand grip for single subject (patient 4) versus control group. The display is as in (A). Plots of the size of task-related brain activation for main effects of left hand grip for each subject in the control group, and patient 4, for (C) right supplementary motor area (x = 18, y = -12, z = 54), and (D) ipsilateral (contralesional) deep central sulcus (x =-16, y = -26, z = 54).

6.3.4 Correlation with outcome in stroke subgroups

Patients were grouped according to the site of the infarct, IC (n=8), MCA (n=6), and pons (n=4). The overall vector of recovery within each subgroup was determined. The first principle components (overall outcome scores) accounted for 78.3% (IC), 80.3% (MCA) and 82.3% (pons) of the variance in each data set.

There were no positive correlations with outcome in any of the subgroups. However, there were significant negative correlations with outcome across all subgroups (table 6.4 and figure 6.4). Regions in which activity correlated negatively with outcome were distributed bilaterally, in primary motor cortex (M1), primary somatosensory cortex (S1), dorsolateral premotor cortex (PMd), ventrolateral premotor cortex (PMv), SMA, pre-SMA, both rostral and caudal parts of CMA, posterior parietal cortex, prefrontal regions, superior temporal gyrus and sulcus, insula cortex, middle and superior occipital gyri, cerebellar hemispheres (lobules V, VI, VIIB, VIIIB, CrI) and vermis. Negative correlations in thalamic activation with outcome were seen only in the MCA group, and in caudate activation, only in the pontine group.

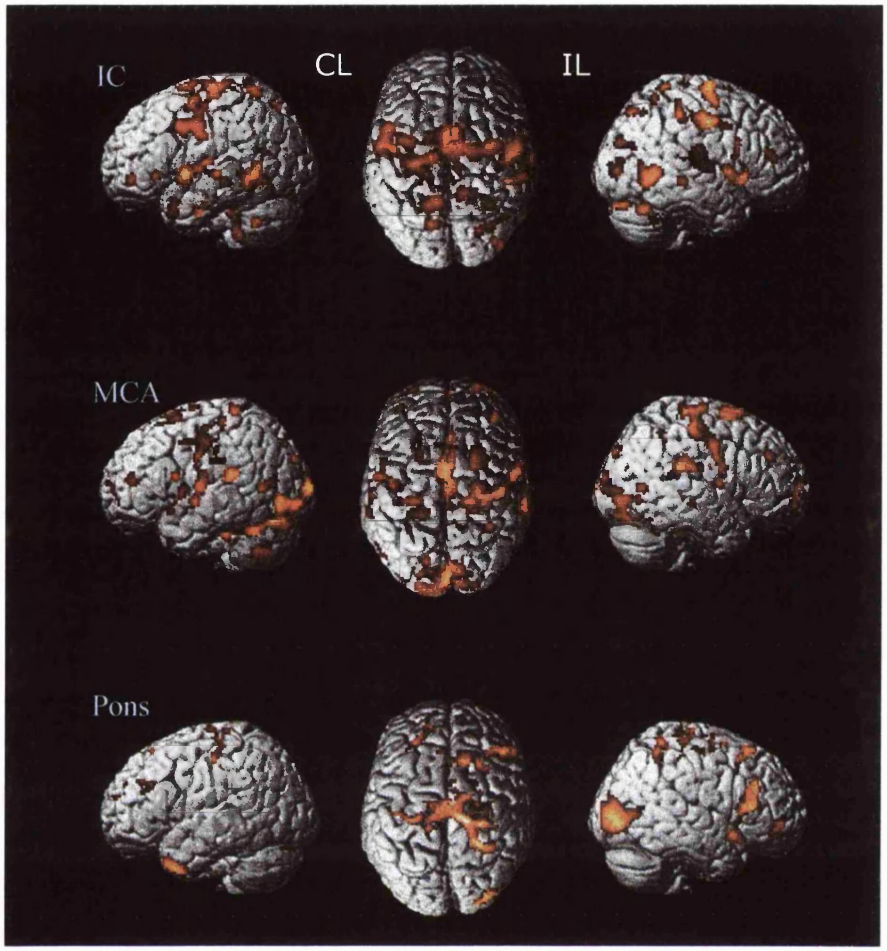


Figure 6.4 SPM{t}s representing voxels for in which there is a negative (linear) correlation between outcome and task related BOLD signal within different stroke subtypes (IC = internal capsule infarcts, MCA = middle cerebral artery infarcts, Pons = pontine infarcts). Results are surface rendered onto a canonical brain. The brain is shown (from left to right) from the left side, from above (left hemisphere on the left), and from the right. All voxels are significant at $p < 0.05$, corrected for multiple comparisons across whole brain.

Table 6.4 *Negative correlations with outcome within stroke sub-groups*

	Internal Capsule (n=8)							Middle Cerebral Artery territory (n=6)							Pons (n=4)						
	Talairach coordinates in MNI space					Correlation Analysis		Talairach coordinates in MNI space					Correlation Analysis		Talairach coordinates in MNI space					Correlation Analysis	
Region	Side	x	y	z	Z-value	r^2	p-value	Side	x	y	z	Z-value	r^2	p-value	Side	x	y	z	Z-value	r^2	p-value
Central Sulcus (M1)	I	42	-18	44	>8.0	0.79	0.003	I	30	-28	72	>8.0	0.78	0.019	I	28	-28	66	6.58	0.77	0.1
	C	-56	-4	40	>8.0	0.79	0.003	I	40	-24	50	>8.0	0.71	0.034	C	-28	-26	64	6.85	0.75	0.1
	C	-48	-10	50	6.77	0.53	0.041	C	-26	-30	60	7.33	0.91	0.003	C	-32	-26	56	>8.0	0.77	0.1
	C	-34	-28	52	5.73	0.6	0.025	C	-52	-8	48	5.95	0.72	0.033							
Postcentral gyrus/sulcus (S1)	I	20	-44	72	6.47	0.7	0.009	I	22	-38	66	5.26	0.8	0.017	I	24	-46	56	>8.0	0.84	0.08
								C	-38	-26	48	5.75	0.79	0.018	I	16	-34	72	5.66	0.88	0.06
								C	-40	-16	44	6.53	0.88	0.005							
PMd	I	34	-10	64	>8.0	0.84	0.001	I	24	-16	74	>8.0	0.54	0.049	I	22	-14	56	6.85	0.8	0.1
	C	-38	-2	58	>8.0	0.71	0.009	I	24	4	70	>8.0	0.6	0.048	I	20	-22	72	5.87	0.86	0.08
								C	-26	-12	50	5.11	0.77	0.022	C	-12	-18	76	6.13	0.78	0.1
								C	-54	2	46	6.62	0.79	0.018							
PMv	I	42	10	18	7.44	0.82	0.002	I	56	0	22	6.28	0.84	0.011							
	C	-52	6	40	>8.0	0.48	0.049														
SMA	I	12	-10	76	>8.0	0.59	0.026	I	2	-18	62	>8.0	0.8	0.016	I	8	-18	56	>8.0	0.77	0.1
	C	-8	-4	74	7.47	0.71	0.008	C	-2	0	64	>8.0	0.64	0.046	C	-4	-20	62	>8.0	0.81	0.1
	M	0	-8	68	>8.0	0.71	0.008														
Pre-SMA	M	0	2	68	>8.0	0.58	0.028	I	4	22	56	6.08	0.98	<0.001	I	10	26	58	6.19	0.77	0.1
															C	-6	30	58	6.72	0.77	0.1
CCZ	I	10	-6	48	>8.0	0.85	0.001	I	4	-2	46	>8.0	0.65	0.038							
	C	-2	-12	48	6.14	0.56	0.032														
RCZ	I	6	14	34	5.27	0.58	0.027														
	C	-10	26	36	6.5	0.79	0.003														
	M	0	6	44	6.82	0.67	0.012	M	0	18	36	>8.0	0.67	0.05							
Superior Parietal Cortex	I	24	-76	52	6.56	0.62	0.019														
	C	-14	-70	56	6.82	0.62	0.02														
Inferior Parietal Cortex	I	66	-20	20	7.73	0.81	0.002	I	64	-30	28	>8.0	0.65	0.047							

Table 6.4 continued

	I	58	-32	48	7.39	0.62	0.019	C	-62	-24	40	5.97	0.68	0.043				
	I	44	-64	50	6.02	0.51	0.048											
	C	-50	-62	24	5.53	0.51	0.047											
Intraparietal Sulcus	I	32	-72	52	6.34	0.77	0.004	I	34	-50	50	6.75	0.66	0.05				
	C	-24	-56	50	5.99	0.61	0.021											
Middle Frontal Gyrus (BA9/46)								I	38	40	32	6.93	0.68	0.042	I	46	30	26	7.71	0.81	0.1
															C	-24	36	28	>8.0	0.85	0.07
Inferior Frontal Gyrus (BA45)	C	-46	46	-4	7.25	0.61	0.021								I	50	26	10	6.56	0.85	0.08
Inferior Frontal Gyrus (BA44)															I	46	20	10	>8.0	0.86	0.07
Inferior Frontal Sulcus	I	52	16	-6	7.16	0.62	0.02														
Superior Temporal Gyrus	I	64	-8	6	7.15	0.59	0.025								I	50	12	-10	7.71	0.93	0.03
	C	-62	-6	2	>8.0	0.6	0.023														
Superior Temporal Sulcus	I	62	-32	-6	6.38	0.77	0.004	I	54	-24	-6	5.96	0.95	0.001							
	C	-54	-24	0	7.08	0.73	0.007	C	-52	-6	-2	7.76	0.66	0.05							
Middle Temporal Gyrus	I	52	-54	2	>8.0	0.89	0.001														
	C	-60	-52	-2	>8.0	0.65	0.016								C	-42	16	-36	>8.0	0.79	0.1
Insula Cortex	I	40	20	-2	6.25	0.56	0.033														
	C	-48	2	0	>8.0	0.71	0.009								C	-26	30	0	7.29	0.89	0.05
Middle Occipital Gyrus	I	42	-88	2	6.9	0.88	0.001	I	44	-80	-4	7.8	0.76	0.025	I	38	-62	4	7.18	0.95	0.02
Cerebellum (Cr I)	I	14	-84	-22	>8.0	0.72	0.008	C	-38	-82	-20	5.81	0.72	0.031							
	I	44	-64	-28	7.33	0.75	0.005														
	C	-48	-54	-40	7.38	0.78	0.004														
	C	-8	-86	-20	6.55	0.55	0.036														
	C	-28	-62	-32	6.01	0.56	0.027														
Cerebellum (VI)	C	-28	-36	-32	6.1	0.78	0.004	I	16	-60	-12	>8.0	0.66	0.048							
								C	-26	-72	-18	>8.0	0.91	0.003							
Cerebellum (V)								I	26	-30	-24	6.23	0.84	0.011							
Cerebellum (VIIB)								C	-46	-58	-48	7.58	0.66	0.048							
Cerebellum (VIIIB)	C	-24	-42	-50	7.58	0.7	0.009														
Cerebellar Vermis	M	2	-62	-32	5.72	0.59	0.026	M	-2	-66	-20	>8.0	0.97	<0.001							
								M	4	-80	-32	7.5	0.78	0.019							
Thalamus (ventral posterolateral)								C	-16	-22	8	6.14	0.98	<0.001							
Thalamus (mediodorsal)								I	2	-6	8	5.13	0.76	0.024							
Caudate															I	20	16	14	7.15	0.88	0.06

Regions in which there is a negative correlation between outcome and task related activation across stroke subtypes. Coordinates represent voxels significant at $p < 0.05$, corrected for multiple comparisons across whole brain volume. The correlation coefficient (r^2) and corresponding p-value for the correlation analysis are also given.
I = ipsilesional, C = contralesional, M = midline, PMd = dorsolateral premotor cortex, SMA = supplementary motor area, CCZ = caudal cingulate sulcus, RCZ = rostral cingulate sulcus, DLPFC = dorsolateral prefrontal cortex.

6.3.5 Correlation with outcome across all stroke patients

I performed a regression analysis between task related activation and an overall outcome score (first principal component, 81.2% of variance), as well as each of the individual outcome scores, across the whole patient group. I found no significant positive correlation with task related activity. As in the subgroup analyses, there was a significant negative linear correlation with outcome across patients (table 6.5, figures 6.5 and 6.6). Thus, the regions which are increasingly likely to be activated by patients with poorer outcome include bilateral PMd, cingulate sulcus and dorsolateral prefrontal cortex, ipsilesional SMA and pre-SMA, ipsilesional insula cortex, bilateral (but more extensively contralesional) cerebellar hemispheres and vermis, together with a number of regions in close proximity to sensorimotor cortex bilaterally. In the ipsilesional hemisphere the regions showing this negative correlation were in central sulcus (z-coordinates 52 and 40), but in the contralesional hemisphere, they included not only central sulcus (z-coordinates 30 and a large cluster from z = 44 to 68), but also postcentral gyrus and postcentral sulcus.

There were no significant correlations between task related activity and the second and third principal components (6.7% and 3.7% of variance, respectively) of the outcome scores across patients.

When examining for the same correlation post hoc using single outcome scores as the independent variable, similar results were obtained (table 6.6), particularly with SMA (bilateral or ipsilesional), sensorimotor cortex (bilateral or contralesional), and cerebellum (bilateral or contralesional).

Table 6.5 *Negative correlations with overall outcome across all patients*

Region	Side	Talairach coordinates in MNI space			Z-value	Correlation analysis	
		x	y	z		r^2	p-value
Central sulcus	C	-54	-8	36	4.41	0.67	<0.0001
	I	38	-26	52	4.02	0.6	0.0001
	I	52	-12	40	4.22	0.64	<0.0001
Posterior central sulcus	C	-38	-24	48	4.53	0.69	<0.0001
Postcentral gyrus	C	-22	-42	66	4.59	0.70	< 0.0001
	C	-62	-20	30	4.25	0.64	<0.0001
Precentral gyrus (BA 4/6)	C	-18	-18	64	4.16	0.63	<0.0001
Dorsolateral premotor cortex	C	-24	-14	52	4.21	0.64	<0.0001
	I	28	4	64	4.43	0.67	<0.0001
Caudal cingulate sulcus (CCZ)	I	6	-8	52	4.98*	0.76	<0.0001
	C	-8	2	62	4.73	0.72	<0.0001
Rostral cingulate sulcus (RCZ)	C	-10	52	-4	3.89	0.58	0.0001
Supplementary motor area (SMA)	I	4	-12	64	4.23	0.64	<0.0001
	C	-2	-2	70	4.18	0.63	<0.0001
Pre-SMA	I	8	20	52	4.55	0.69	<0.0001
Insula	I	44	22	2	4.28	0.65	<0.0001
Dorsolateral prefrontal cortex	I	34	34	28	4.03	0.60	0.0001
	C	-34	34	20	4.01	0.60	0.0001
Cerebellum (Cr I)	C	-32	-76	-24	4.98*	0.76	<0.0001
Cerebellum (VIIB)	C	-20	-66	-42	4.93	0.75	<0.0001
Cerebellum (VI)	C	-26	-30	-32	5.11*	0.77	<0.0001
Cerebellar Vermis	M	4	-62	-38	4.66	0.71	<0.0001

Regions in which there is a negative correlation between outcome and task related activation across all stroke patients. Coordinates represent peak voxels within significant cluster ($p < 0.05$, corrected for multiple comparisons across whole brain volume). *Voxels significant at $p < 0.05$, corrected for multiple comparisons across whole brain volume. The correlation coefficient (r^2) and corresponding p-value for the correlation analysis are also given.

I = ipsilesional, C = contralesional, M = midline, PMd = dorsolateral premotor cortex, CCZ = caudal cingulate sulcus, RCZ = rostral cingulate sulcus, DLPFC = dorsolateral prefrontal cortex.

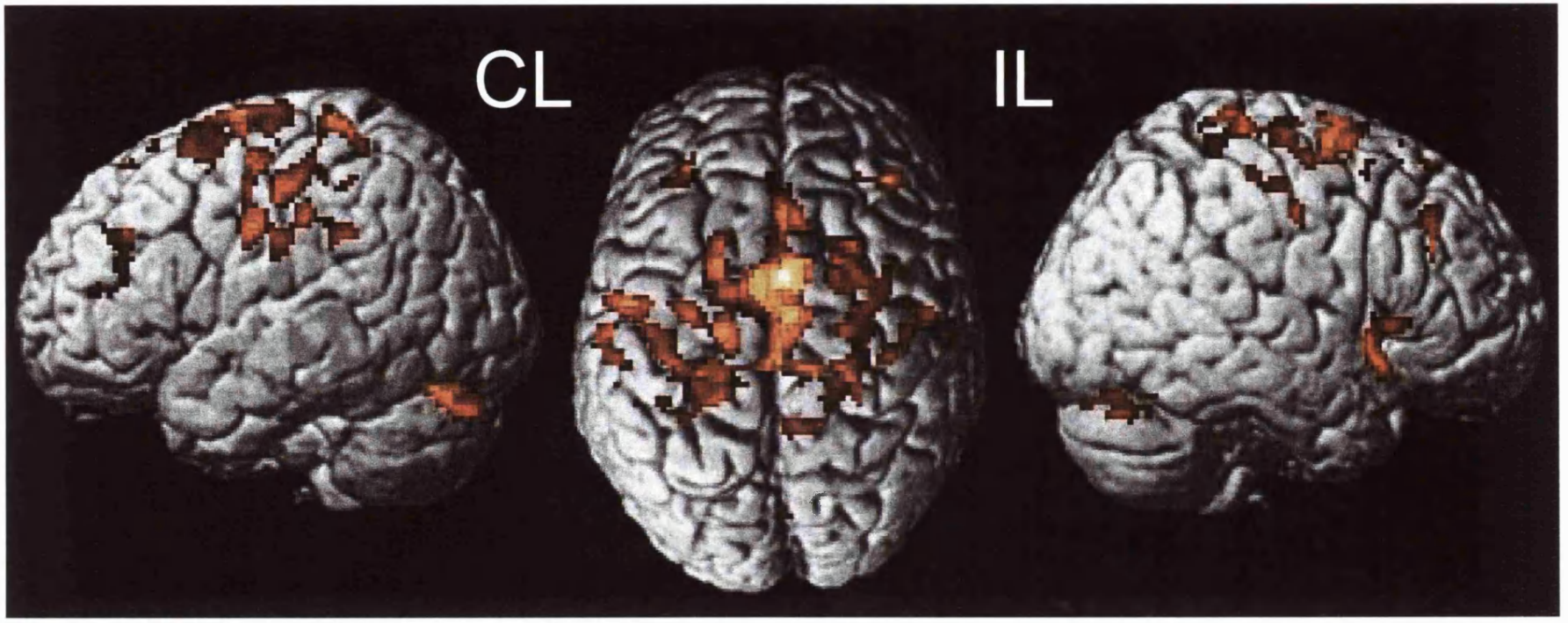
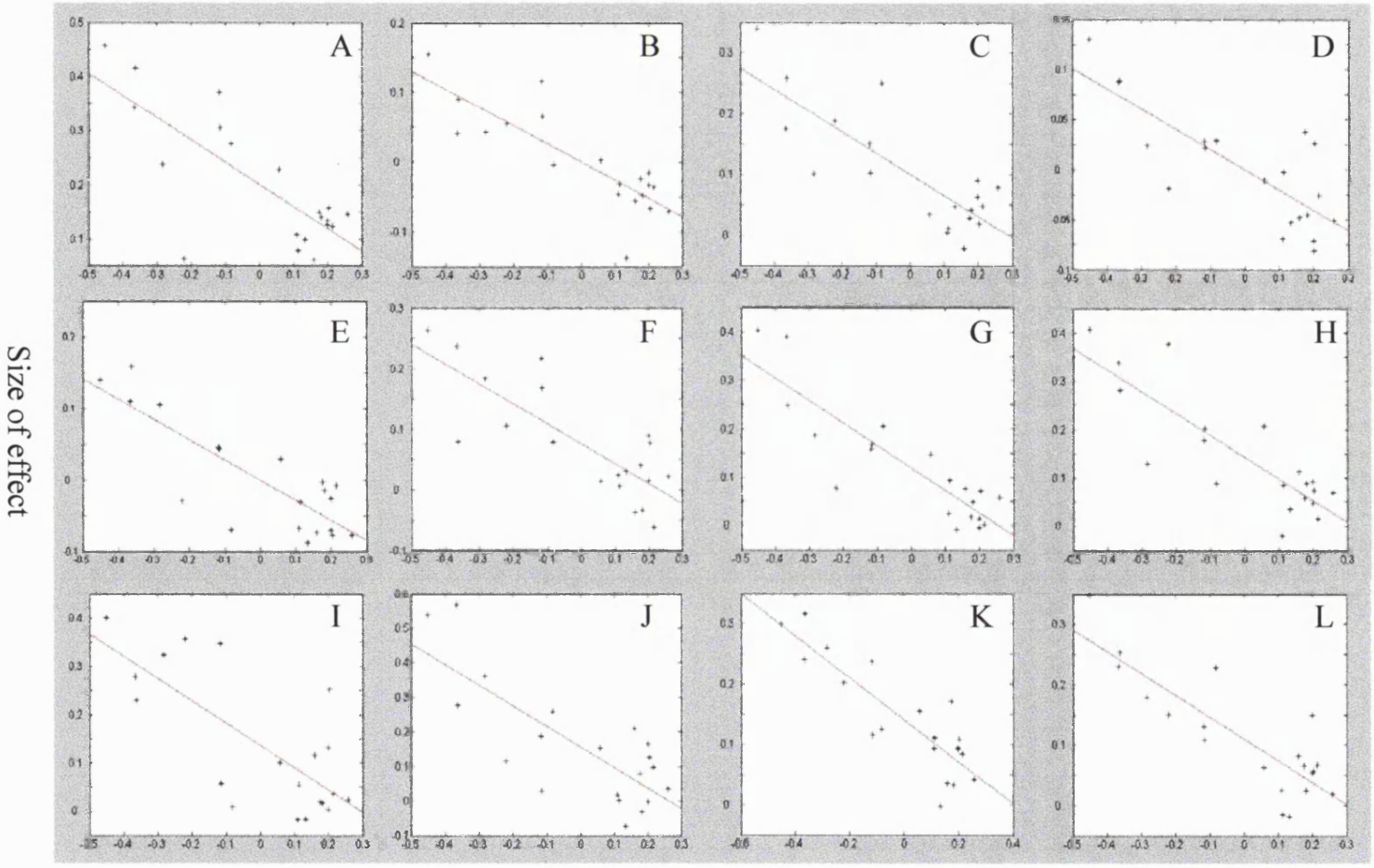


Figure 6.5 SPM{t}s representing regions in which there is a linear negative correlation between outcome and task related BOLD signal across all stroke. Results are surface rendered onto a canonical brain. The brain is shown (from left to right) from the left side, from above (left hemisphere on the left), and from the right. IL = ipsilesional, CL = contralesional. All clusters are significant at $p < 0.05$, corrected for multiple comparisons across whole brain.

Figure 6.6


A
B
C
D
E
F
G
H
I
J
K
L
Size of effect
Overall Outcome Score (normalised)

Legend to figure 6.6 Illustrative plots of size of activation against relative outcome score (normalised) for all chronic stroke patients. Higher values of recovery score represent better outcome. The correlation coefficient and the associated p-value are given in brackets. (A) Ipsilesional primary motor cortex (x = 38, y = -26, z = 52) (r^2 = 0.60, p = 0.0001); (B) contralesional inferior M1 (r^2 = 0.67, p<0.0001); (C) ipsilesional lateral dorsal premotor cortex (r^2 = 0.64, p<0.0001); (D) contralesional lateral dorsal premotor cortex (r^2 = 0.67, p<0.0001); (E) contralesional posterior central sulcus (primary sensory cortex) (r^2 = 0.69, p<0.0001); (F) ipsilesional insula cortex (r^2 = 0.65, p<0.0001); (G) contralesional cerebellum (VI) (r^2 = 0.77, p<0.0001); (H) ipsilesional cerebellum (VI) (r^2 = 0.71, p<0.0001); (I) ipsilesional supplementary motor area (r^2 = 0.64, p<0.0001); (J) contralesional supplementary motor area (r^2 = 0.63, p<0.0001); (K) ipsilesional caudal cingulate sulcus (r^2 = 0.76, p<0.0001); (L) contralesional caudal cingulate sulcus (r^2 = 0.72, p<0.0001).

6.3.6 Linear and non-linear correlations between BOLD signal and force of handgrip

The statistical parametric maps derived from the parameter estimates for the first (linear), and second order (non-linear) polynomial expansions of the handgrip force, demonstrate voxels in which the BOLD signal exhibits a higher order relationship to force of hand grip. In the control group, linear increases with either hand were seen in ipsilesional sensorimotor cortex, cerebellar vermis, contralesional cerebellar hemisphere (inferior cerebellum during dominant hand grip, superior cerebellum for non-dominant hand grip), and contralesional ventroposterior lateral thalamus. No second order relationships were consistently demonstrated with use of the non-dominant hand, but were seen with dominant hand use. Positive second order effects were seen in ipsilesional rostral cingulate sulcus and intraparietal sulcus, contralesional superior frontal sulcus, caudal cingulate sulcus and dorsolateral prefrontal cortex, and bilaterally in insula cortex.

Table 6.6 *Negative correlations with individual outcome scores*

Region	Barthel	Rankin	OPS	ARAT	MI (UL)	MI (LL)	10m walk	NHPT	Grip
SMC	B	B	C	C	B		B	B	B
PMd			I	C		I	C		C
PMv									
SMA	B	I		B	B	B		I	I
CMA		I					B		B
PPC				C	C				C
PFC		C		B	B				B
Insula		I			I				
Temporal Lobe	C	I	C	B	C				
V5			I	I					
V1				B	B	B		B	B
Cerebellum		B	B	B	B	C	C	C	C
Vermis			M	M		M	M		
Putamen									
Thalamus									

A significant negative correlation between task related activity and each outcome score across patients is indicated for each region (I =ipsilesional, C = contralesional, B = bilateral, M = midline) if cluster significant at $p < 0.05$, corrected for multiple comparisons across whole brain.

SMC = sensorimotor cortex, PMd = dorsolateral premotor cortex, PMv = ventrolateral premotor cortex, SMA = supplementary motor area, CMA = cingulate motor area, PPC =posterior parietal cortex, PFC = prefrontal cortex. OPSS = Orpington Prognostic Stroke Scale, ARAT = Action Research Arm Test, MI = Motricity Index, NHPT = nine hole peg test, UL = upper limb, LL = lower limb.

A negative second order effect was seen in a cluster with peak voxel in right medial orbitofrontal cortex.

No consistent first or second order effects in the patient group as a whole could be identified that were different to the control group, but there were significant differences within the patient group (table 6.7). There was a negative correlation between the first order increase in BOLD signal as a function of increasing grip force and outcome in ipsilesional central sulcus (deep), postcentral gyrus, PMd, middle temporal gyrus, contralesional cerebellum, and bilateral intraparietal sulcus. In other words these regions were more likely to exhibit a linear increase in activity in response to increasing grip force in patients with poorer outcome. Furthermore a number of regions were identified in which there was more likely to be a negative second order relationship (an 'inverted-U' shape) between BOLD signal and increasing hand grip force in patients with poorer outcome. These regions include a number of medial motor areas, (SMA and CMA), ipsilesional precentral gyrus (BA 4/6), postcentral gyrus, superior frontal sulcus (BA 6/8), and cerebellum (lobule VI), contralesional superior temporal sulcus and putamen, and bilateral intraparietal sulcus.

Table 6.7 *Relationship between task related brain activation, peak hand grip force and outcome*

Region	Side	Talairach coordinates in MNI space			Z-value
		x	y	z	
(a) Force related linear increases in BOLD signal in less well recovered patients					
Central sulcus (deep)	I	32	-32	40	3.91
Postcentral gyrus	I	58	-28	44	3.9
PMd	I	22	-2	58	4.1
Intraparietal sulcus	I	40	-48	42	3.57
	C	-34	-48	60	4.03
Middle temporal gyrus	I	54	-68	4	5.4*
Cerebellum (VI)	C	-12	-80	-18	4.91
Cerebellar vermis	M	4	-84	-28	4.42
(b) Force related negative second order effects on BOLD signal in less well recovered patients					
Precentral gyrus (BA 4/6)	I	36	-12	46	4.91
Postcentral gyrus	I	32	-40	64	4.11
Superior frontal sulcus (BA 6/8)	I	32	10	58	4.99
SMA	I	8	0	66	3.76
CCZ	I	6	-6	44	4.32
Intraparietal sulcus	I	36	-54	56	3.9
	C	-32	-38	40	5.21*
STS	C	-50	2	-22	4.01
Putamen	C	-20	-8	8	3.78
Cerebellum (VI)	I	26	-46	-32	4.19

Regions in which there is a differential relationship between BOLD signal and peak force exerted during a hand grip in patients with different degrees of recovery. (a) Regions that are more likely to exhibit linear increases in BOLD signal as a function of increasing hand grip force in less well recovered patients. (b) Regions in which a negative second order ('inverted-U') relationship is more likely to be seen in less well recovered patients. Coordinates represent peak voxels within significant cluster ($p < 0.05$, corrected for multiple comparisons across whole brain volume). *Voxels significant at $p < 0.05$, corrected for multiple comparisons across whole brain volume.

I = ipsilesional, C = contralesional, M = midline, PMd = dorsolateral premotor cortex, SMA = supplementary motor area, CCZ = caudal cingulate sulcus, STS = superior temporal sulcus.

6.4 Discussion

These results demonstrate that in patients who have suffered from hemiparetic stroke, there are significant increases in task related activation in a number of brain regions, over and above those seen in the normal population performing the same task. Furthermore, by carefully measuring a number of outcome variables in each patient, I have demonstrated for the first time, a relationship between task-related activations and outcome. Specifically, patients with poorer outcome are more likely to recruit a number of regions during the performance of a motor task. It is important to state that I have been able to make such an inference from the data as a result of the fact that each patient was performing an equivalent motor task, as discussed in chapter 4. That my strategy succeeded in controlling for effort across subjects is supported by the results of the VAS ratings for effort completed by each subject.

An important aspect of the experimental design is the way in which outcome has been defined. A single outcome rating was created for each patient, accounting for 81.2% of the variance across all outcome scores and for a similar amount across subgroups. However, I also performed post-hoc regression analyses between task related activity across the whole group and each individual (normalised) outcome score. There is clearly some variation, but an inverse relationship between activation and outcome is consistently seen in very similar brain regions, particularly SMA, sensorimotor cortex, and cerebellum (table 6.6).

6.4.1 Task related activations in patients

A wide range of motor related regions were activated during the task in all patients, as has been seen in other studies (Chollet *et al.*, 1991; Weiller *et al.*, 1992; Weiller *et al.*, 1993; Cramer *et al.*, 1997; Seitz *et al.*, 1998; Cao *et al.*, 1998). In this study, patients with poorer outcome were more likely to activate a number of brain regions, including premotor and parietal cortex, SMA, and cerebellum, Furthermore, the relationship between activation in these regions and outcome was linear. In explaining these results it is useful to consider recent advances in the understanding of the functional neuroanatomy of the premotor (both lateral and medial wall) regions (Strick, 1988; Dum and Strick, 1991; Dum and Strick, 1996). The notion of a hierarchical motor system with premotor regions at the top and primary motor cortex acting as the final common pathway for the central control of movement has been challenged by observations made supporting the existence of separate parallel motor networks involving cerebellar and basal ganglia afferents and their projections to cortical motor regions via ventrolateral thalamus (Strick, 1988). Outputs from deep caudal cerebellar nuclei influence the arcuate (lateral) premotor area, those from deep rostral cerebellar nuclei influence the primary motor cortex and those from globus pallidus interna influence the SMA (Strick, 1988). Furthermore each of these cortical motor regions receives input from different parietal regions. Thus, Strick (Strick, 1988) proposed that these circuits are parallel and independent, with interactions between them occurring at the level of the cortex. Subsequent experiments have indicated that there is a high degree of similarity between the corticospinal projections from the hand regions of M1, SMA and CMA (Dum and Strick, 1996;

Rouiller *et al.,* 1996), thus providing the substrate whereby a number of motor networks acting in parallel could generate an output to the spinal cord necessary for movement. The implication is that damage in one of these networks could be compensated for by activity in another, thus explaining the recruitment of these regions seen in recovered stroke patients.

However, these new findings indicate that bilateral recruitment of such regions occurs in stroke patients with poorer, not better, outcome. Recent work has demonstrated that the projections from M1 to the motorneurons in the spinal cord are more numerous and exert a greater excitatory effect than those from SMA (Maier *et al.,* 2002), suggesting that in the situation where SMA projection to spinal cord motor neurons augment or substitute for those from M1, the functional consequences may fall short of fractionated finger movements. Thus in the patient group, in those with return of fractionated finger movements, cortico-motorneuronal input from M1 is presumably preserved, hence a 'normal' activation pattern, as input from other motor circuits is less important or suppressed. But in those in whom the M1 cortico-motorneuronal input is lost or impaired, there is greater reliance on other parallel motor circuits to generate an alternative input to the spinal motorneurons, resulting in increased task related activation. However, because of the nature of these projections (Maier *et al.,* 2002), functional recovery is incomplete. Support for the importance of M1 cortico-motorneuronal projections for full recovery comes from the relationship between preserved TMS induced motor evoked potentials to the hand and recovery of hand function post stroke (Heald *et al.,* 1993; Pennisi *et al.,* 1999; Cruz *et al.,* 1999).

Task related brain activation was *not* different to the normal population in six patients with good outcome (table 6.3). It might be expected that the initial severity of their motor impairment was mild, or that they had partial internal capsule lesions. But this is not the case. Of the six, four had marked weakness of wrist and finger extensors (grade 2 or less on the MRC grade of muscle power) at presentation, and three had striatocapsular infarcts. Post hoc analysis found no correlation between task related activity in the chronic phase and the amount of motor improvement from the time of presentation.

In attempting to reconcile my results to those of previous studies, I suggest that patients in some previously reported studies may not have been fully recovered as judged by a larger number of outcome measures such as I used in this study. Indeed, three out of six patients in one study (Chollet *et al.*, 1991) described residual slowness of finger movements. The results in these patients were similar to those of my patients with residual motor deficit. More recent studies have taken account of differential outcomes in patients studied. Johansen-Berg *et al.*, (2002a) described more bilaterally distributed motor cortex activations in patients with greater impairment, and Feydy *et al.*, (2002) suggested that non M1-lesioned patients with better outcome seemed more likely to focus task related brain activations towards a relatively normal pattern over 3 separate fMRI sessions. Both these sets of results would be consistent with my findings.

6.4.2 Task related activity in contralesional M1

A great deal of the motor system is represented bilaterally (Tanji *et al.*, 1988). Indeed, in the control group, task related activations, other than in contralateral

sensorimotor cortex and ipsilateral superior cerebellum were largely bilateral. Thus, recruitment of parallel motor networks involving premotor regions and their connections with cerebellum and parietal cortex is likely to be bilateral, which is confirmed by my results. Recruitment of contralesional (ipsilateral) M1 however cannot be explained in this way. Anatomical studies suggest that both direct (corticospinal) and indirect (corticoreticulospinal) pathways from ipsilateral M1 end in projections to axial and proximal stabilising muscles rather than hand muscles (Brinkman and Kuypers, 1973; Carr *et al.,* 1994). However, repetitive TMS to M1 results in errors in both complex and simple motor tasks using the ipsilateral hand (Chen *et al.,* 1997) suggesting that ipsilateral M1 may play a role in planning and organisation of hand movement. Some functional imaging studies have reported activation in ipsilateral M1 (Cramer *et al.,* 1999), whilst others have observed task related deactivations in ipsilateral M1 (Allison *et al.,* 2000). In the control group I observed only task related deactivation of ipsilateral M1, particularly in younger subjects, although this deactivation diminished as a function of increasing age. In the patient group, activation in ipsilateral (contralesional) M1, including the hand region, was observed in five less well recovered patients (table 6.3). I did not observe movement or surface EMG activity of the unaffected arm or hand to explain these activations. These results are similar to those in which TMS to contralesional M1 elicited MEPs in the affected arm only in stroke patients with poorer outcome (Turton *et al.,* 1996; Netz *et al.,* 1997), findings which were thought to call into question the functional role of contralesional M1. Furthermore, in a group of stroke patients shown to activate contralesional M1 during movement of the affected hand, single pulse

TMS to M1 did not affect performance of simple reaction time finger movements (Johansen-Berg *et al.*, 2002a). TMS to contralesional PMd however, did prolong reaction times, suggesting that contralesional PMd, but not M1, is contributing significantly to motor recovery in those patients. Interestingly however, I observed a negative correlation between task related activity and recovery not in the hand region of contralesional M1, but caudally in postcentral gyrus, and in ventral central sulcus (z = 36). Furthermore this latter region was situated deep in central sulcus, possibly in the putative area 4p (Geyer *et al.*, 1996). TMS to the motor hot spot for hand muscles would not affect these parts of the sensorimotor cortex. I conclude that parts of contralesional M1 do generate a motor output to the affected hand. However, as with premotor regions, this recruitment occurs only in patients with a significant deficit, possibly as a result of loss of ipsilesional M1 cortico-motoneuronal projections, in which a dependency on alternative motor projections has developed.

6.4.3 Task related activity in ipsilesional M1

Shifts in the peak ipsilesional (ipsilesional) sensorimotor task related activations in post stroke patients have been observed in previous studies (Weiller *et al.*, 1993; Pineiro *et al.*, 2001). All the patients in this study activated ipsilesional M1, but only four activated ipsilesional M1 hand region over and above the control group. That might occur if they were gripping with greater force, but the absolute force exerted was less than in well recovered patients (since the proportion of MVC used was the same across the group). Across the group, I observed a negative correlation between outcome and task related activity in two parts of

ipsilesional M1. The first was situated medial to the hand region, deep in central sulcus (x = 38, y = -26, z = 52), possibly representing the putative area 4p (Geyer *et al.*, 1996), and the second ventral to the hand region (x = 52, y = -12, z = 40). The more dorsal of these is located within the region activated by the control group, so that hand grip in less well recovered patients was associated with greater, rather than new, activation in this region. The more ventral region was beyond that activated by the control group, suggesting a ventral shift in task related M1 activation in some less well recovered patients. A similar enlargement in the motor output zone was observed as a consequence of degeneration of the corticospinal tract (Kew *et al.*, 1994), possibly as a result of loss of recurrent inhibition onto surrounding pyramidal cells (Ghosh and Porter, 1988).

Changes in cortical maps as a consequence of lesions to the corticospinal pathway may enable the lesioned brain to take advantage of considerable redundancy within the somatotopy of M1 (in that a number of combinations of pyramidal cells may produce the same movement (Sanes and Donoghue, 2000) to generate an output to the spinal cord via an intact portion of the pyramidal tract. One might also speculate that plastic changes in somatotopic representations in SMA, CMA, and premotor regions may occur (as a consequence of a lesion, or driven by a therapeutic intervention), resulting in stronger connections with different regions of M1 (i.e. with alternative representations of the hand region) in order to generate an output to the spinal cord. Some studies using transcranial magnetic stimulation have demonstrated increases in motor cortex representation of hand muscles in stroke patients correlating with the degree of clinical improvement after a period of rehabilitation (Traversa *et al.*, 1997). This finding is similar to the therapy

driven increases in premotor cortex activation (as demonstrated with fMRI), also associated with clinical improvement in stroke patients (Johansen-Berg *et al.*, 2002b). These changes occurring in single subjects may indeed facilitate functional improvement. Clearly studies using both techniques in the same subjects will be required. Such studies, together with thorough measures of outcome, will help us to interpret the contribution of each technique.

Increases in task related brain activation as a result of increased attention to a simple motor task have been observed in a number of motor regions, including SMA, cingulate cortex, insula, post-central sulcus and deep central sulcus (putative area 4p) (Johansen-Berg and Matthews, 2002). It could be argued therefore, that some of my results are due to patients with poor outcome needing to pay more attention to the task. I attempted to control for attention, by incorporating visual feedback, to which all subjects were asked to attend. However, it is possible that increased attention to the task is a key strategy in the less well recovered patient, increasing activation in a number of motor regions, thereby providing a substrate for activity dependent plastic changes in somatotopic representations within the motor system and consequently alternative motor pathways. This is very similar to the notion of patients accessing latent motor engrams described by Luria (1963), and Meyer (1973). Evidence that attention can modulate motor performance can be seen in the effect that attending to a neglected limb has in patients with unilateral neglect (Robertson *et al.*, 1997) and may also explain the beneficial effect that d-amphetamine is said to have on motor function (in the context of motor practice) in both animals and humans (Feeney, 1997).

6.4.4 Subgroup analysis

Patients with MCA infarcts recovered less well than the other two groups overall, but within groups there was significant variability, as reflected by the differential activation patterns. However, there were no significant differences in the correlation analyses between groups. It is likely that the stroke patients in this study were relatively homogeneous in terms of the relevant neuroanatomical structures affected by their infarct. None of the subjects had damage to the hand region of M1. A prime determinant of outcome may be the degree to which direct M1 cortico-motoneuronal projections are disrupted, with less recovery and greater recruitment of alternative motor circuits in those with some loss of these projections. One might hypothesise that in a cohort of patients with complete disruption of M1 cortico-motoneuronal projections, recruitment of premotor circuits involving SMA, CMA and lateral premotor cortex would facilitate recovery, such that a positive correlation between outcome and task related activation of these regions would be observed. This idea is partially supported by the observation of bilateral motor related activation in both PMd and SMA, but not M1, in patients with good outcome from middle cerebral artery territory infarcts involving the cortical hand area (Seitz *et al.*, 1998).

6.4.5 Alterations in linear and non-linear BOLD responses to increasing force

Using single cell recordings in macaque monkeys, both linear and non-linear force related firing rates in cortical neurons have been reported (Evarts *et al.*, 1983). Studies in humans have demonstrated linear force related responses in ipsilesional sensorimotor cortex, caudal cingulate sulcus, SMA and contralesional cerebellum

with functional imaging (Dettmers *et al.*, 1995) and non-linear responses were observed in the control group. These non-linearities have been interpreted as either recruitment or saturation effects in different populations of neurons within functionally similar cortical regions. Dettmers and colleagues described an altered relationship between force exerted in a finger press task and regional cerebral blood flow (rCBF) in several stroke patients with incomplete recovery (Dettmers *et al.*, 1997). Ipsilesional sensorimotor cortex activity showed a binomial relationship with force compared to a logarithmic relationship in controls. Furthermore, linear increases in rCBF with increasing force, not seen in controls, were observed in SMA and parietal cortex. These observations provide further evidence to support a role for alternative motor networks in patients regaining some motor function after stroke. I have extended this observation, and describe differential linear and non-linear force related responses across the spectrum of recovery in a number of parallel motor networks. Although it is difficult to interpret these results in terms of their relationship to the recovery process, it is clear that after focal damage the motor system adapts not only in terms of what structures are engaged but also in how those structures respond.

6.4.6 Conclusions

I have correlated task related activation and outcome after stroke for the first time in a functional imaging study. I have been able to do this because of detailed measurement of a variety of aspects of functional outcome in each patient. This approach has revealed an inverse relationship between task-related brain activation and outcome, which may at first seem counter intuitive. I do not suggest

that this relationship is causative. Instead I suggest that the potential for recovery is limited, at least in part, by the degree of damage to the ipsilesional M1 cortico-motoneuronal projection to the spinal cord, which is critical for fractionated finger movements. Subsequent recovery is likely to be a function of neuronal reorganisation within remaining motor related areas to generate alternative input to the spinal motor neurons. In short, greater reorganisation occurs in those with greatest need. In that sense the changes that I have described are likely to be driving the functional gains that have been made in each individual. These results further our understanding of the likely mechanisms underlying functional recovery after stroke.

Chapter 7

NEURAL CORRELATES OF MOTOR RECOVERY AFTER STROKE:

A LONGITUDINAL FMRI STUDY

7.1 Introduction

In the last chapter, I demonstrated that chronic stroke patients with less than full recovery are more likely to activate a number of primary and non-primary motor regions over and above the normal population. In those with complete recovery, motor-related activation patterns were indistinguishable from the normal population. However, the dynamic process of changing brain activation patterns and the relationship of this process to recovery is less well understood. Two studies in which patients were scanned early after stroke, and then again some months later, have been discussed in chapter 2 (Marshall *et al.*, 2000; Calautti *et al.*, 2001a). However, the dynamic evolution of task related activations remains unclear. I postulated that by scanning patients at more frequent time points,

particularly during periods of greatest behavioural change (i.e. early after stroke), and by obtaining detailed outcome measures relating to different aspects of the recovery process, I would be able to identify changes in motor-related brain activation patterns occurring not as a function of time after stroke, but as a function of recovery.

7.2 Materials and methods

7.2.1 Subjects

Patients were recruited from the acute stroke and rehabilitation services at the National Hospital for Neurology and Neurosurgery, Queen Square, London. All patients had suffered from first-ever ischaemic stroke resulting in weakness of at least wrist and finger extensors, and hand interossei to ≤ 4+ on the Medical Research Council scale, for at least 48 hours after onset of symptoms. Exclusion criteria consisted of (1) carotid artery occlusion or stenosis ≥ 70%; (2) language or cognitive deficits sufficient to impair co-operation in the study.

The age-matched control group was recruited from the volunteer database at the Wellcome Department of Imaging Neuroscience. They reported no history of neurological illness or psychiatric history and were not taking regular medication. Neurological and rheumatological examinations were normal in all control subjects. .

All patients and control subjects were right handed according to the Edinburgh handedness scale (Oldfield, 1971). Full written consent was obtained from all subjects in accordance to the Declaration of Helsinki. 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 Trust, London.

7.2.2 Behavioural evaluation

The behavioural evaluations were identical to those carried out in chapter 6 and described in detail in chapter 4 and appendix I. A full evaluation was carried out at each scanning session. Thus for each patient nine measures of recovery were recorded at each assessment, creating nine recovery curves (one each for Barthel, Rankin, OPS etc.) per patient. Each patient's set of recovery curves was normalised (giving unit variance and zero mean) and a principal component analysis was performed on the data set of each patient. The first principal component was taken as the representative recovery curve across sessions for each patient. This recovery curve was used to examine for correlations between task-related activations and recovery for each patient.

7.2.3 Motor paradigm

Patients were first scanned at 10-14 days post stroke onset, then weekly for at least the next 4 weeks. Subsequent scans were then carried out at least 3 and 6 months post stroke onset and in some cases 12 months. Control subjects (two using the dominant hand and two using the non-dominant hand) were scanned at weekly intervals for 4 weeks, then again 2 and 5 months later. During scanning subjects performed a dynamic isometric hand grip task using the magnetic resonance imaging compatible manipulandum with visual cueing and feedback as described previously (chapter 5). Patients used their impaired hand. Maximum

voluntary contraction (MVC) was assessed with subjects in a lying position prior to each scanning session. During a continuous scanning session, subjects performed the paced isometric dynamic hand grips in blocks of 20 seconds, alternating with 20 seconds rest. A total of 10 blocks of hand grip, and 10 rest blocks were performed per session. Target forces and rates of hand grip were constant within each twenty second block, but were presented in two different forms within each session. In hand grip task A, the target force was 20% of MVC performed at 40% of the patient's maximum rate, as measured *on the day of scanning.* Thus in a recovering patient, the absolute force generated per hand grip would increase in task A over sessions, but would remain at 20% of their MVC on that day. In hand grip task B, the target force was 40% of MVC performed at 40% of the patient's maximum rate as measured *at the first session.* Thus in a recovering patient, the absolute performance parameters (target force and rate) were unaltered in task B across sessions. Five blocks of each task (A) and task (B) were performed in each scanning session in a pseudorandomised, counterbalanced order. My primary interest was in task A, but task B was included in order to address the hypothesis that maintaining absolute task parameters across sessions will lead to overestimation of session effects.

All patients performed the motor task outside the scanner in order that they might be observed for the presence of associated movements or mirror movements. To aid this assessment, patients held two identical hand grip manipulanda, one in each hand, during the performance of repetitive hand grip with the affected hand. These simultaneous recordings from both hands enabled us to detect true mirror movements (Nelles *et al.,* 1998). In addition surface electromyogram (EMG)

electrodes were positioned on biceps, triceps, and latissimus dorsi bilaterally, to detect more proximal muscle activation.

7.2.4 Data acquisition

A Siemens VISION system (Siemens, Erlangen, Germany), operating at 2 T, was used to acquire both T_1-weighted anatomical images (1 x 1 x 1.5 mm voxels) and T_2*-weighted MRI transverse echo-planar images (EPI) (64 x 64 3 x 3 mm^2 pixels, T_E = 40ms) with blood oxygenation level dependent (BOLD) contrast. Each echoplanar image comprised forty eight 1.8 mm thick contiguous axial slices taken every 3mm, positioned to cover the whole cerebrum. A total of 116 volumes were acquired continuously during each session, with an effective repetition time (T_R) of 3.649 seconds per volume. The first six volumes were discarded to allow for T_1 equilibration effects.

7.2.5 Image analysis

Pre-processing of control and patient scans was carried out as previously described in chapters 5 and 6. Statistical analysis was performed in two stages. Once again, all hand grips were defined as a single event type, and modelled as delta functions. Hand grips during task A and B were included as separate covariates. These covariates were convolved with a canonical synthetic haemodynamic response function, and were used in a general linear model (Friston *et al.*, 1995a) together with a single covariate representing the mean (constant) term over scans. The parameter estimates for each covariate resulting from the least mean squares fit of the model to the data were calculated and

statistical parametric maps of the t statistic (SPM{t}) resulting from linear contrasts of each covariate (Friston *et al.*, 1995a) were generated and stored as separate images for each subject.

The first experimental question related to whether a correlation exists between the voxel-wise task-related changes in BOLD signal and the degree of recovery across sessions in each patient. This question was addressed using one multiple-session fixed effects model per patient, with covariates representing handgrips during task A and task B entered separately for each session. I examined for voxels in which there is a linear correlation between the recovery score and the parameter estimates for the covariates representing the main effects of hand grip (task A or task B) across sessions. The specific contrasts across each covariate were weighted according to the mean corrected recovery scores (either positively or negatively) appropriate for each patient, in order to generate an appropriate SPM{t} representing a 'recovery map' (i.e. brain regions in which task-related activations correlate (either positively or negatively) with the degree of recovery across sessions) for each patient. For significant voxels, the correlation coefficient for the plot of parameter estimate against recovery for each subject, together with the corresponding *p*-value, was calculated.

The second experimental question related to whether task-related activation in specific regions of the brain would correlate with recovery across subjects in a group analysis. This was addressed in a second stage of analysis, for which the data comprised the pooled parameter estimates for the specific linear combination of covariates representing the 'recovery maps' for each patient. For these group analyses, the images of patients with left sided infarcts (right hand weakness)

were flipped about the mid-sagittal line, such that all subjects were considered to have right sided infarcts. Thus, contrast images for each subject were entered into a one sample t-test, and SPM{t}s generated.

The third experimental question concerned the direct comparison between 'recovery maps' for tasks A and B. I contend that in a longitudinal study of this kind, the notion of maintaining the same motor task across sessions is best dealt with by maintaining %MVC as target force, rather than maintaining absolute force. Thus, using the same multiple-session fixed effects models described above, a combination of weighted contrasts (derived from the mean corrected recovery scores) across covariates for tasks A and B was used, representing the direct comparison of recovery-related changes in task-related brain activation across sessions for both tasks. Previous attempts at characterising longitudinal changes have often done so in terms of shifts in task-related activation between contralesional and ipsilesional sensorimotor and premotor cortices (Marshall *et al.*, 2000; Feydy *et al.*, 2002). Therefore I limited the comparison of tasks A and B to these regions using a small volume correction. The regions of interest were defined as spheres of radius 20 mm centred on coordinates x = ±38, y = -26, z = 56, representing hand M1 (Fink *et al.*, 1997).

It was not possible to generate 'recovery maps' for the control subjects as no change in function occurred, but I was able to examine for time (session) effects across each subject. The maps of session effects were generated using the same single subject, multi-session fixed effects model described above, examining for linear changes in task-related activation as a function of session (time) for each

subject. Only indirect comparisons were possible between patient 'recovery maps' and control 'session maps'.

The resulting SPM{t}s were thresholded at $p < 0.05$, corrected for multiple comparisons across whole brain. All SPM{t}s were transformed to the unit normal Z-distribution to create a statistical parametric map (SPM{Z}).

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).

7.3 Results

7.3.1 Clinical data

The control group was aged between 27 and 67 years (mean age 47.3 years), and comprised two male and two female subjects. Eight stroke patients were recruited (range 29 to 71 years, mean age 52.8 years). Patient characteristics are listed in table 7.1. Patients attended for between six to ten sessions (mean 8.0), and controls attended for six sessions each. The site of cerebral infarction was determined from the T_1-weighted structural MRI (figure 7.1) Five patients had right-sided infarcts, three had left-sided infarcts. Three patients had infarcts isolated to the internal capsule (two posterior, one anterior), one patient had an infarct in the corona radiata, three patients had pontine infarcts, and one patient had an infarct in the striatocapsular region with extension to the insula cortex as a result of a branch middle cerebral artery occlusion. No patient had damage to the

primary motor cortex. Incomplete sensory deficit, described as a slight reduction in sensation compared to the unaffected side, was detected in two patients.

All patients received both inpatient and outpatient post stroke rehabilitation therapy appropriate to their clinical needs. The outcome measures demonstrated improvement in performance in all patients (table 7.2). See Appendix II for full details of recovery scores. The first principal component of each patients' set of recovery curves accounted for between 78.2% and 90.5% of the variance within each data set (table 7.2), and each was taken as the overall patient specific recovery curves for the purposes of correlation analysis (figure 7.2).

7.3.2 Behavioural results

All controls and patients were able to perform the task adequately. No patients displayed mirror movements at bedside observation. When performing the motor paradigm outside the scanner there was no evidence of mirror movements or surface EMG activity in biceps, triceps or latissimus dorsi muscles on the side opposite the affected hand. However, patients 1 and 2 did exhibit synergistic wrist flexion, but not elbow flexion, nor shoulder adduction during rehearsal of the motor paradigm at sessions 1 and 2. A 100 mm visual analogue scale (VAS) (where 0 = 'no effort' and 100 = 'maximum effort') was completed by patients after practicing task A (target grip force set at 20% of MVC on day of scanning) prior to each scanning session. No significant differences existed in the perceived effortfulness of the task across sessions. There was no correlation between the rating for effort and the overall recovery score for each patient (table 7.2).

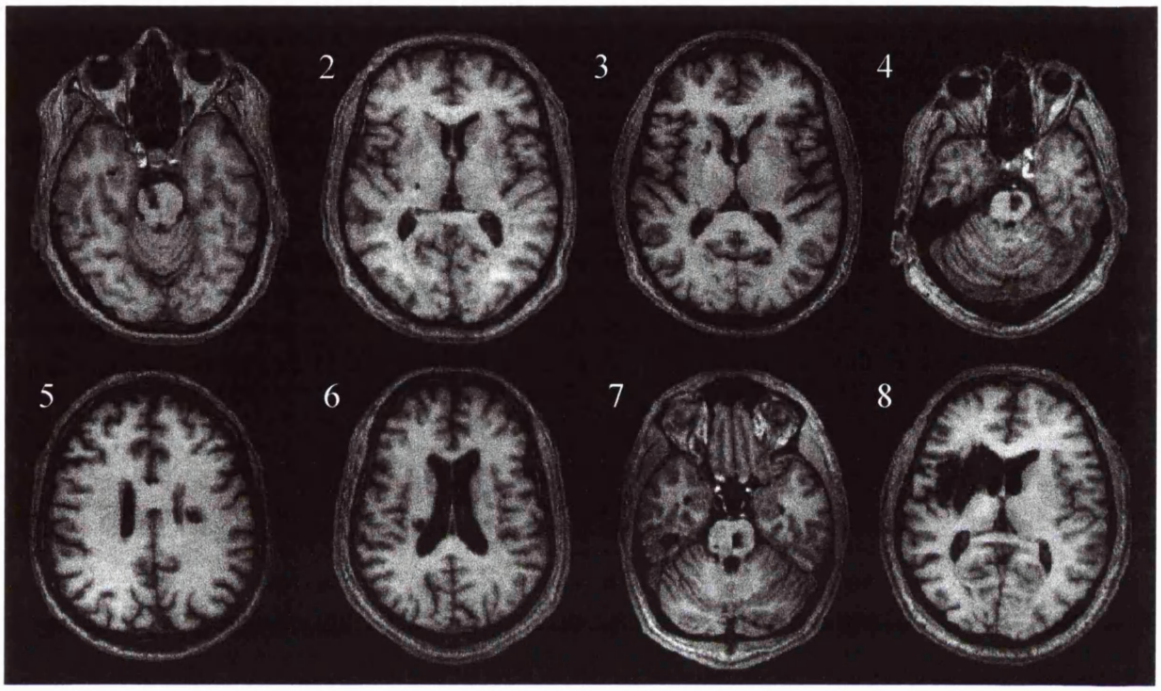


Figure 7.1 Axial structural $T1$-weighted MRI scans at the level of maximum infarct volume for each patient. The front of the brain is displayed at the top, and the right side on the left.

Table 7.1 *Patient characteristics*

Patient	age	sex	affected hand	Site of lesion	Number of fMRI sessions	PMH	Medication
1	53	F	L	R pons	6	hypertension	aspirin, metoprolol
2	56	M	L	R IC (posterior)	7	nil	aspirin
3	63	M	L	R IC (anterior)	7	hypertension	aspirin, atenolol, amlodipine, simvastatin
4	71	M	R	L pons	10	IHD, COPD	aspirin
5	52	M	R	L corona radiata	6	hypertension	aspirin, atenolol, ramipril
6	60	M	L	R IC (posterior)	10	hypertension	aspirin, bendrofluazide
7	29	M	R	L pons	10	nil	aspirin
8	38	M	L	R striatocapsular region and insula cortex	8	nil	aspirin

M = male, F = female, R = right, L = left, IC = internal capsule, MCA = middle cerebral artery, NIDDM = non-insulin dependent diabetes mellitus, IHD = ischaemic heart disease, COPD = chronic obstructive airways disease

Table 7.2 *Early and late outcome scores for stroke patients*

	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5		Patient 6		Patient 7		Patient 8		contribution of each measure to 1st principal component (mean (SD))
	early (11)	late (200)	early (10)	late (197)	early (9)	late (207)	Early (14)	late (174)	early (14)	Late (193)	early (10)	late (191)	Early (13)	late (370)	Early (10)	Late (312)	
Barthel	12	20	19	20	19	20	12	20	19	20	18	20	11	20	14	20	11.4% (0.4)
Rankin	3	1	2	1	2	1	4	2	2	1	4	1	4	1	4	1	11.4% (0.3)
Orpington Prognostic Scale	2.4	1.6	2	1.6	2	1.6	3.2	2	2	1.6	2.4	1.6	3.6	1.6	2.8	1.6	11.3% (0.7)
Action Research Arm Test	33	57	56	57	56	57	53	57	56	57	54	57	13	57	28	57	10.6% (1.4)
Grip Strength (% unaffected side)	41.2	88.6	48.1	101.2	71.3	93.1	47.9	98.7	91.8	103.3	71.3	104.9	3.2	100.9	49.1	106.9	10.6% (1.1)
Motricity Index - upper limb	67	100	93	100	93	100	72	92	93	100	77	100	62	100	73	100	11.1% (0.7)
Motricity Index - lower limb	73	100	92	100	92	100	64	92	92	100	73	100	40	100	92	100	11.4% (0.3)
9 Hole Peg Test (% unaffected side)	3.8	74.3	35.9	75.4	62.7	108.2	30.1	86.7	90.3	102.8	29.9	78.7	0	78.1	0	67.1	11.0% (1.1)
10 metre walk (m/s)	0.45	1.33	0.4	1.4	1.01	1.6	0	1.15	1.95	2.25	0	1.02	0	1.68	0	1.4	11.2% (0.6)
Sensory Loss	no	no	face/ hand	hand	no	no	no	no	no	no	hand	hand	no	no	no	no	
Neglect*	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
Mirror movements	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	
†% variance explained by 1st principal component	87.50%		83.30%		87.40%		78.50%		78.20%		84.30%		90.50%		83.60%		
‡Correlation between VAS score for effort and overall recovery scores	r^2=0.07, p=ns		r^2=0.11, p=ns		r^2=0.04, p=ns		r^2=0.21, p=ns		r^2=0.02, p=ns		r^2=0.06, p=ns		r^2=0.02, p=ns		r^2=0.16, p=ns		

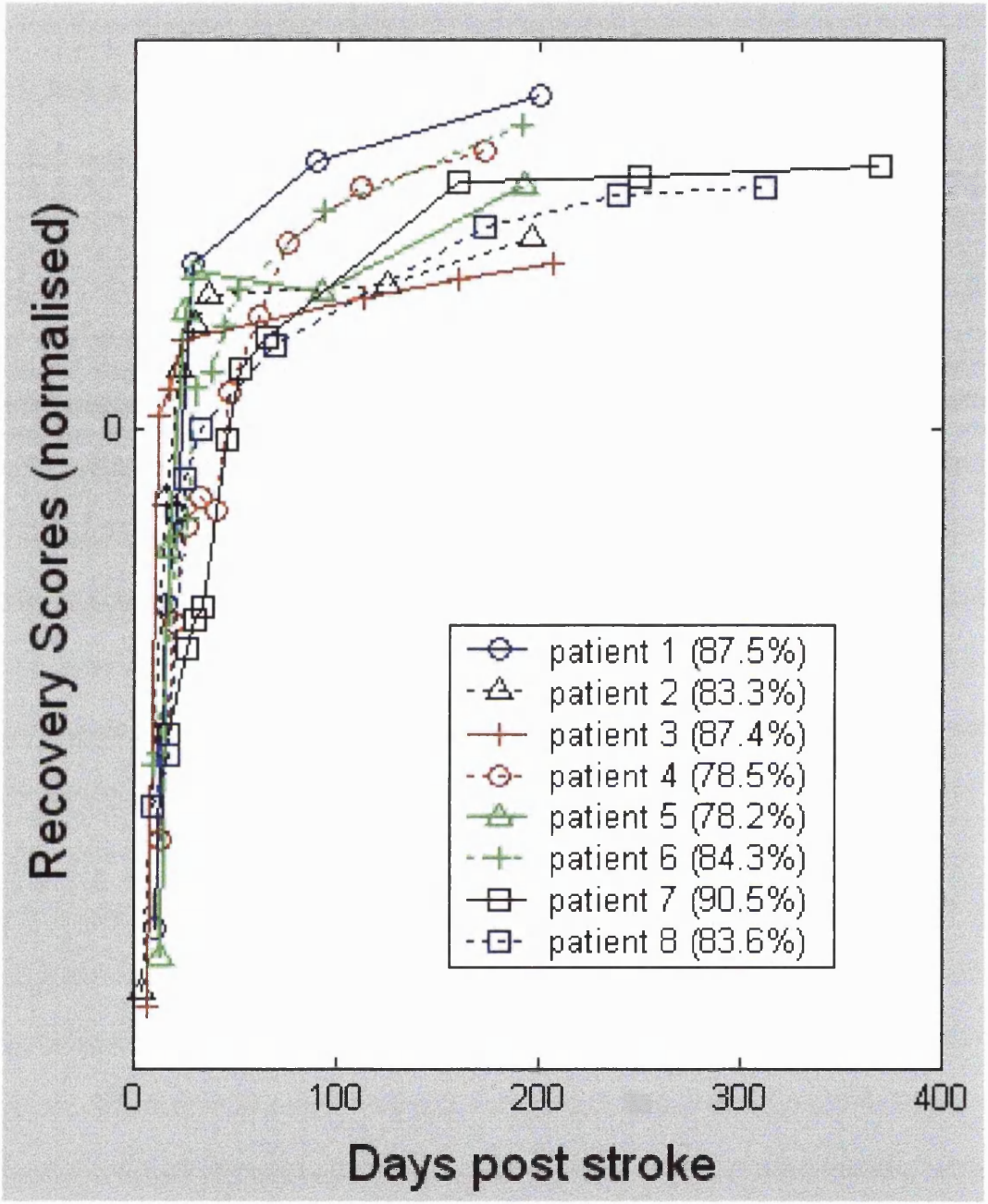


Figure 7.2 Plots of normalised overall recovery scores for each patient across sessions. Each patient had nine separate performance scores recorded at each fMRI session (Rankin, Barthel, OPSS etc.), creating nine recovery curves per patient. The overall recovery score represents the first principal component of a principal component analysis of these nine recovery curves. The amount of variance in the original data set explained by the first principal component is given in brackets. There is no scale on the y-axis because the scores are normalised, and have no meaningful absolute value, only relative value.

7.3.3 Changes in motor-related brain activation as a function of recovery

The neural correlates of the hand grip task were not explicitly examined in this study, but are described chapter 5. I was primarily interested in the 'recovery maps' for task A, which will now be described. Brain regions are described as either ipsilesional (i.e. contralateral to the moving hand) or contralesional (i.e. ipsilateral to the moving hand). A negative correlation between task-related increases in brain activation and recovery across sessions (i.e. recovery-related decreases in task-related activations) was seen in a number of brain regions in all eight patients (figure 7.3 and table 7.3). A negative correlation was particularly common in motor-related regions, in particular ipsilesional primary motor cortex (M1) (five patients), contralesional M1 (four patients), dorsal and ventral premotor cortex (PMd and PMv - seven and five patients respectively), supplementary motor area (SMA - six patients), cingulate motor regions (CMA - four patients), and cerebellum (seven patients). In addition, recovery-related decreases were observed in both inferior and superior parietal cortex, as well as intraparietal sulcus (seven patients) and prefrontal regions (six patients). Brain activations in both temporal and occipital lobe have been observed with this hand grip task (chapter 5). Interestingly, task-related activity in these regions also decreased with recovery in some subjects, as well as in subcortical structures such as thalamus and basal ganglia structures.

In the group analysis recovery-related decreases in task-related activation across sessions were seen throughout ipsilesional M1, from z = 36 to 52, and in inferior contralesional M1, as well as in anterior and posterior dorsolateral premotor cortex bilaterally (BA 6 and BA 8), contralesional PMv, and ipsilesional SMA-

proper, pre-SMA, prefrontal cortex (superior frontal sulcus) and caudal cingulate sulcus (figure 7.4 and table 7.4). In addition, these decreases were seen in parietal, temporal and occipital lobes, as well as thalamus and globus pallidus.

A positive correlation between task-related activation and recovery across sessions (i.e. recovery-related increases in task-related activations) was seen in some brain regions in four patients (with lesions involving pons (two), anterior internal capsule, and corona radiata). No such increases were seen in the other four patients with lesions involving posterior internal capsule (two), pons, or striatocapsular region with extension to the insula cortex. The severity of deficit at presentation was matched between the two groups. A number of regions demonstrated recovery-related increases in task-related activations across sessions (table 7.3), including ipsilesional PMv and anterior cingulate cortex, and cerebellum (in two patients each). Three patients showed increases in ipsilesional superior temporal sulcus in the mid to posterior segment. In the group analysis, no brain regions demonstrated a significant recovery-related increase in activation, suggesting no such consistent pattern across patients

Table 7.3a *Single subject analysis (patients 2,3,5,6): Regions in which activations correlate linearly with recovery*

	Patient 2							Patient 3							Patient 5							Patient 6						
		Talairach coordinates in MNI space				Correlation analysis			Talairach coordinates in MNI space				Correlation analysis			Talairach coordinates in MNI space				Correlation analysis			Talairach coordinates in MNI space				Correlation analysis	
Region	Side	x	y	z	Z-value	r^2	p-value	Side	x	y	z	Z-value	r^2	p-value	Side	x	y	z	Z-value	r^2	p-value	Side	x	y	z	Z-value	r^2	p-value
(a) Negative correlation																												
Central sulcus (M1)	I	36	-22	60	4.99	0.8	<0.01	I	58	-12	44	6.99	0.9	<0.01	I	-18	-28	66	4.33*	0.6	0.05	I	40	-22	66	>8.0	0.8	0.01
	I	50	-16	58	4.81	0.7	0.03	C	-58	-8	44	>8.0	0.8	0.01	C	20	-26	72	5.75	0.6	0.04	I	40	-28	52	6.75	1	<0.01
PMd	I	54	0	50	6.49	0.7	0.03	I	18	-14	78	6.68	0.7	0.02								I	38	16	54	5.22	0.6	0.05
anterior PMd (BA 6/8)								C	-46	14	50	5.96	0.9	<0.01	I	-26	16	44	6.36	0.6	0.05							
								C	-16	8	68	5.73	0.7	0.02	C	22	20	62	6.26	0.9	<0.01							
Caudal PMv								I	52	4	28	5.37	0.7	0.01	I	-52	-6	16	4.42*	0.7	0.05	I	58	0	18	4.21*	0.8	0.01
								C	-48	12	34	6.31	0.9	<0.01														
								C	-50	-2	28	5.02	0.6	0.05														
Rostral PMv (BA 44)	I	50	16	14	4.88	0.6	0.03																					
SMA	I	6	0	62	5.84	0.7	0.01	C	-8	-4	72	6.17	0.8	<0.01	I	-4	-18	64	4.45*	0.7	0.04							
Pre-SMA	I	10	14	64	5.79	0.6	0.03																					
	C	-6	8	60	5.01	0.7	0.03																					
Cingulate sulcus	I	12	16	42	5.52	0.6	0.04	C	-6	30	24	7.11	0.9	<0.01														
								C	-12	-18	44	6.19	0.8	0.01														
Anterior cingulate gyrus								I	8	12	26	6.81	0.8	0.01														
Inferior parietal cortex	I	62	-26	44	5.65	0.9	<0.01	I	58	-32	48	6.89	0.9	<0.01	I	-50	-68	38	5.07	0.8	0.02	C	-34	-68	34	3.80*	0.6	0.05
	I	50	-62	44	4.97	0.9	<0.01	C	-48	-68	34	<8.0	0.9	<0.01														
	C	-52	-20	38	4.82	0.7	0.02	C	-58	-42	48	7.54	0.9	<0.01														
Intraparietal sulcus	I	46	-42	44	5.51	0.8	<0.01	I	28	-68	56	7.39	0.9	<0.01								I	28	-68	50	6.97	0.8	0.01
	C	-34	-42	56	5.95	0.9	<0.01																					
Superior parietal cortex								I	14	-78	48	7.02	0.9	<0.01								I	36	-40	66	5.73	0.8	0.01
								C	-16	-78	48	<8.0	0.9	<0.01														
								C	-10	-56	72	5.32	0.9	<0.01														
Superior temporal gyrus								C	-50	20	-14	6.66	0.9	0								C	-66	-24	10	4.92	0.9	<0.01
Superior temporal sulcus								I	60	-56	14	6.76	0.8	0.01	C	36	-50	26	5.51	0.8	0.02							
								C	-58	-42	8	7.59	1	<0.01														
Insula	I	42	-4	-10	4.79	0.8	<0.01															I	40	0	2	4.56*	0.9	0.01
Parietal operculum (S II)	I	60	-18	26	6.17	0.8	<0.01	I	58	-26	16	5.99	0.9	<0.01	C	52	-32	20	5.49	0.8	0.02							

Table 7.3a *continued*

								C	-54	-28	22	6.01	0.8	0.01										
Prefrontal cortex	I	46	54	8	6.82	0.6	0.03	I	40	42	28	5.83	0.7	0.01										
	I	56	22	36	6.2	0.6	0.04	C	-42	32	36	> 8.0	0.9	<0.01										
	I	54	24	0	7.09	0.8	0.01	C	-54	28	20	6.51	0.9	<0.01										
	C	-52	38	18	5.05	0.7	0.05																	
Striate cortex	I	18	-84	12	5.16	0.8	<0.01	I	10	-62	16	6.76	0.9	<0.01	I	-12	-70	10	5.62	0.6	0.05	I	10	-72	-10	5.32	0.9	<0.01
								C	-6	-72	12	6.54	0.9	<0.01	C	24	-70	10	5.54	0.6	0.05							
Middle Occipital Gyrus	I	28	-96	6	>8.0	0.9	<0.01	I	50	-78	10	5.57	0.9	<0.01								I	32	-92	4	6.32	0.8	0.02
	C	-24	-94	20	6.13	0.9	<0.01	C	-36	-74	16	7.39	0.9	<0.01								C	-34	-76	10	5.19	0.9	0.01
Cerebellum	I-VIIIA	26	-38	-48	5.41	0.7	0.02	I-VIIIB	30	-40	-44	7.53	0.9	<0.01								I-VI	20	-58	-24	5.76	0.8	0.02
	I-V	12	-56	-10	5.02	0.6	0.04	I-Crl	28	-78	-32	6.06	1	<0.01								C-VI	-22	-66	-20	6.16	0.8	0.01
								C-Crl	-30	-88	-20	6.36	1	<0.01								C-Crl	-24	-72	-30	5.03	0.7	0.04
								C-Crl	-48	-66	-36	5.68	0.8	<0.01														
Cerebellar Vermis	VIIB	4	-72	-26	5.36	0.9	<0.01	VIIIA	-2	-64	-32	5.14	0.6	0.04	V	-4	-60	0	5.43	0.8	0.02	VI	0	-62	-28	4.93	0.8	0.01
Thalamus								I	6	-2	2	7.67	0.9	<0.01														
								I	6	-22	4	6.21	0.8	<0.01														
								C	-16	-16	16	6.11	0.8	<0.01														
Caudate								I	12	20	2	4.93	0.7	0.01	C	18	6	16	4.64*	0.9	<0.01							
								C	-14	6	18	5.88	0.7	0.02														
Globus pallidus								I	18	8	-8	5.94	0.8	0.01														
(b) positive correlation																												
PMd								C	-18	-10	76	7.18	0.9	<0.01														
PMv															I	-42	32	42	5.11	0.9	0.01							
Superior parietal cortex								C	-22	-50	58	> 8.0	0.9	<0.01														
Intraparietal sulcus															I	-26	-68	38	5.25	0.8	0.02							
Superior temporal sulcus								I	54	-24	-2	6.64	0.8	<0.01														
								C	-64	-20	-2	5.54	0.6	0.03														
Cerebellum															C-V	22	-50	-24	4.87	0.9	<0.01							

Regions in which there is a correlation between recovery score and task-related activation across all sessions for patients with internal capsule (patients 2,3,6) or corona radiata (patient 5) infarcts. Voxels significant at $p < 0.05$, corrected for multiple comparisons across whole brain volume. *Coordinates represent peak voxels within significant cluster ($p < 0.05$, corrected for multiple comparisons across whole brain volume). The correlation coefficient (r^2) and corresponding p-value (significant at $p < 0.05$) for the correlation analysis are also given. I = ipsilesional, C=contralesional, M = midline, PMd = dorsolateral premotor cortex, PMv = ventrolateral premotor cortex, SMA = supplementary motor area. Roman numerals for cerebellar activations refer to cerebellar lobules (Schmahmann *et al.*, 1999).

Table 7.3b *Single subject analysis (patients 1,4,7,8): Regions in which activations correlate linearly with recovery*

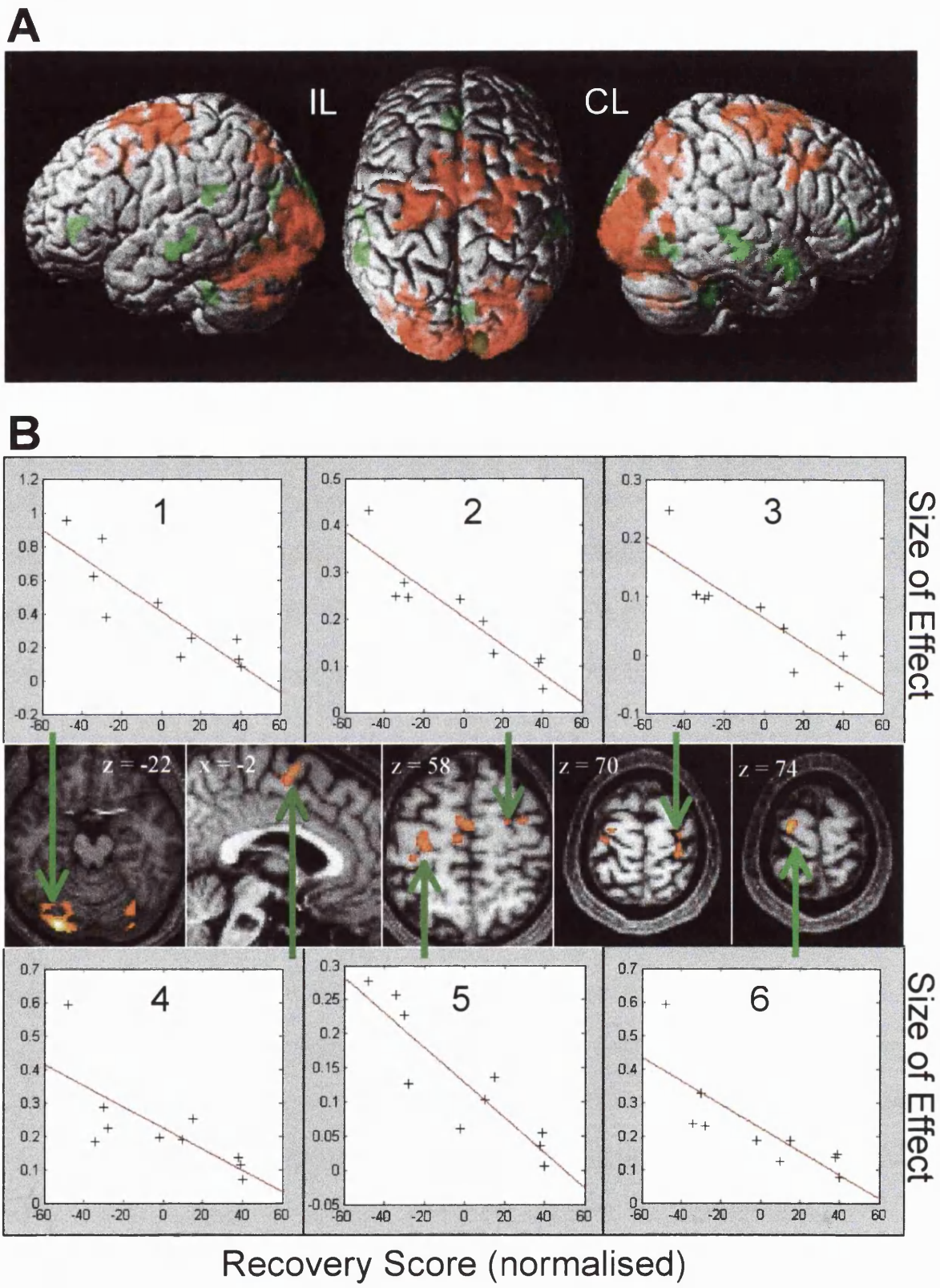
	Patient 1							Patient 4							Patient 7							Patient 8						
		Talairach coordinates in MNI space				Correlation analysis			Talairach coordinates in MNI space				Correlation analysis			Talairach coordinates in MNI space				Correlation analysis			Talairach coordinates in MNI space				Correlation analysis	
Region	Side	x	y	z	Z-value	r2	p-value	Side	x	y	z	Z-value	r2	p-value	Side	x	y	z	Z-value	r2	p-value	Side	x	y	z	Z-value	r2	p-value
(a) Negative correlation																												
Central sulcus (M1)															I	-30	-14	58	5.6	0.8	<0.01	I	22	-34	60	7.19	0.7	0.01
															C	28	-14	70	5.25	0.7	<0.01	I	44	-14	58	6.95	0.9	<0.01
																						C	-40	-26	60	7.7	0.7	0.01
PMd								C	42	0	58	6.18	0.8	<0.01	I	-18	-10	74	6.88	0.6	0.01	I	38	-6	64	5.63	0.9	<0.01
								I	-28	-20	70	5.49	0.6	0.01	I	-28	-8	68	5.14	0.6	0.01	I	24	0	66	5.62	0.9	<0.01
															C	32	-10	68	4.96	0.7	<0.01	C	-26	-12	68	6.16	0.9	<0.01
															C	38	0	58	5.02	0.9	<0.01							
anterior PMd (BA 6/8)								C	24	14	68	5.34	0.5	0.02								I	50	24	42	5	0.8	<0.01
Caudal PMv															C	48	10	34	4.45*	0.8	<0.01	I	42	-2	40	6.76	0.7	0.01
																						C	-52	10	34	5.39	0.8	<0.01
Rostral PMv (BA 44)								C	58	12	4	5.16	0.5	0.03														
SMA								C	10	-2	56	5.11	0.5	0.04	I	-2	-2	60	6.12	0.5	0.02	I	6	-10	70	> 8.0	0.7	0.01
															I	-6	-12	58	5.03	0.9	<0.01	C	-2	-18	64	> 8.0	0.8	<0.01
								C	8	12	68	4.94	0.7	<0.01	C/I	0	10	48	4.50*	0.5	0.03	I	4	22	52	6.53	0.5	0.04
Cingulate sulcus								I	-10	0	46	5.22	0.5	0.03								I	8	0	44	7.7	0.7	0.01
																						C	-8	-12	42	5.61	0.6	0.02
Anterior cingulate gyrus	I	14	54	-4	5.31	1	<0.01																					
Inferior parietal cortex																						I	38	-62	48	7.91	0.9	<0.01
Intraparietal sulcus								C	32	-44	42	5.01	0.5	0.03	C	30	-74	38	6.14	0.7	<0.01	C	-54	-40	42	6.85	0.9	<0.01
															I	-24	-74	42	4.50*	0.4	0.05	I	44	-50	56	7.35	0.9	<0.01
Superior parietal cortex								I	-30	-56	66	5.12	0.7	0.01	C	18	-70	60	4.58*	0.5	0.02	C	-20	-74	52	> 8.0	0.9	<0.01
								C	36	-64	58	4.83	0.6	0.01								I	20	-76	52	> 8.0	0.9	<0.01
Superior temporal gyrus																						C	-56	6	-6	6.63	0.7	0.01
Superior temporal sulcus																						I	62	-6	-16	5.37	0.6	0.02
Insula	C	-32	12	-14	4.18*	0.9	0.02	C	42	18	-2	5.21	0.5	0.02								C	-36	-6	-6	5.07	0.7	0.01
Frontal operculum															C	68	-10	14	5.25	0.5	0.02	C	-64	-32	20	7.62	0.9	<0.01

Table 7.3b *continued*

Prefrontal cortex	C	-42	34	22	5.35	0.8	0.03	C	42	52	20	5.29	0.6	0.01	I	-38	58	-10	5.05	0.5	0.02	I	38	50	22	> 8.0	0.8	<0.01
								I	-34	48	28	5.41	0.7	<0.01								C	-34	48	32	6.12	0.7	0.01
																						C	-32	28	6	7.2	0.9	<0.01
Middle Occipital Gyrus															C	48	-66	4	6.6	0.8	<0.01							
Cerebellum	I-CrI	50	-52	-36	6.13	0.8	0.05								I-CrI	-26	-84	-22	> 8.0	0.8	<0.01	I-VI	12	-86	-22	>8.0	0.9	<0.01
	I-CrII	48	-48	-44	4.97	1	<0.01								I-VI	-20	-66	-18	6.05	0.8	<0.01	C-VI	-16	-72	-18	>8.0	0.8	<0.01
Cerebellar Vermis															VI	-2	-44	-2	5.32	0.8	<0.01	VIIIB	0	-72	-42	> 8.0	0.9	<0.01
															VIIIB	0	-70	-46	4.79*	0.6	0.01							
Thalamus								I	-10	-14	8	5.78	0.5	0.02														
Caudate	I	16	20	-6	6.04	0.8	0.03																					
Putamen	I	22	4	-10	4.83*	0.8	0.03																					
(b) positive correlation																												
M1	I	46	-18	40	5.87	0.8	0.04																					
Caudal PMv	I	52	6	40	5.06	0.8	0.03																					
Parietal operculum (SII)	I	58	-26	26	4.98	1	<0.01																					
Rolandic operculum	I	60	-6	16	5.2	0.8	0.05																					
Superior temporal sulcus	I	58	-20	-8	6.09	0.8	0.03								I	-64	-40	6	6.39	0.8	<0.01							
															I	-52	-18	4	5.64	0.5	0.03							
Prefrontal cortex															C	36	54	24	5.8	0.7	<0.01							
															C	46	20	52	5.23	0.5	0.03							
Anterior Cingulate Gyrus	C	-10	14	30	4.64*	0.8	0.04								I	-2	42	4	5.09	0.5	0.02							
Cerebellum	I-CrII	42	-60	-44	4.56*	0.8	0.03																					
	C-CrII	-24	-40	-44	5.48	0.8	0.04																					
Thalamus	I	16	-14	0	4.51*	1	<0.01																					
	C	-14	-14	0	4.41*	1	<0.01																					

Regions in which there is a correlation between recovery score and task-related activation across all sessions for patients with pontine infarcts (patients 1, 4, 7) and patient with middle cerebral artery territory infarct (patient 8). Voxels significant at $p < 0.05$, corrected for multiple comparisons across whole brain volume. *Coordinates represent peak voxels within significant cluster ($p < 0.05$, corrected for multiple comparisons across whole brain volume). The correlation coefficient (r2) and corresponding p-value (significant at $p < 0.05$) for the correlation analysis are also given. I = ipsilesional, C=contralesional, M = midline, PMd = dorsolateral premotor cortex, PMv = ventrolateral premotor cortex, SMA = supplementary motor area. Roman numerals for cerebellar activations refer to cerebellar lobules (Schmahmann *et al.*, 1999).

Figure 7.3


A
IL
CL
B
1
2
3
4
5
6
z = -22
x = -2
z = 58
z = 70
z = 74
Size of Effect
Size of Effect
Recovery Score (normalised)

Legend to figure 7.3 Results of single subject (patient 7) longitudinal analysis examining for linear changes in task-related brain activations over sessions as a function of recovery. Patient 7 suffered from a left sided pontine infarct resulting in right hemiparesis. (A) Results are surface rendered onto a canonical brain; red areas represent recovery-related decreases in task-related activation across sessions, and green areas represent the equivalent recovery related increases. All voxels are significant at $p < 0.001$ (uncorrected for multiple comparisons) for display purposes. The brain is shown (from left to right) from the left (ipsilesional) side, from above (left hemisphere on the left), and from the right (contralesional). IL = ipsilesional, CL = contralesional. (B) Results are displayed on patients own normalised T_1-weighted anatomical images (voxels significant at $p < 0.05$, corrected for multiple comparisons across the whole brain), with corresponding plots of size of effect against overall recovery score (normalised), for selected brain regions. Coordinates of peak voxel in each region are followed by the correlation coefficient and the associated p-value: (1) ipsilesional cerebellum (x=-26, y=-84, z=-22) (r^2=0.77, p<0.01), (2) contralesional dorsolateral premotor cortex (x=38, y=0, z=58) (r^2=0.85, p<0.01), (3) contralesional M1 (x=28, y=-14, z=70) (r^2=0.74, p<0.01), (4) ipsilesional supplementary motor area (x=-2, y=-2, z=60) (r^2=0.53, p=0.02), (5) ipsilesional M1 (x=-30, y=-14, z=58) (r^2=0.80, p<0.01), (6) contralesional dorsolateral premotor cortex (x=-18, y=-10, z=74) (r^2=0.63, p=0.01).

Legend to figure 7.4 (A) Group 'recovery map': brain regions in which linear reductions in task-related activation across sessions as a function of recovery were consistently detected for the whole group. This represents the random effects group analysis in which the data from individual 'recovery maps' were pooled across all subjects. Images for patients with left sided lesions were flipped about the mid-sagittal line, so that all patients were assumed to have a lesion on the right side, with initial left hand weakness. Results are surface rendered onto a canonical brain. The brain is shown (from left to right) from the left (contralesional) side, from above (left hemisphere on the left), and from the right (ipsilesional). All clusters are significant at $p < 0.05$, corrected for multiple comparisons across whole brain. IL = ipsilesional, CL = contralesional.

Regions that consistently demonstrate recovery related decreases in task-related activation across sessions in all subjects studied longitudinally shown on brain slices, (B) clusters in ipsilesional central sulcus and premotor cortex, (C) clusters in contralesional ventral central sulcus, premotor and prefrontal cortex, (D) supplementary motor area, and pre-supplementary motor area. Activations displayed on canonical brain slices. cs = central sulcus. All clusters significant at $p < 0.05$ (corrected for multiple comparisons across whole brain)

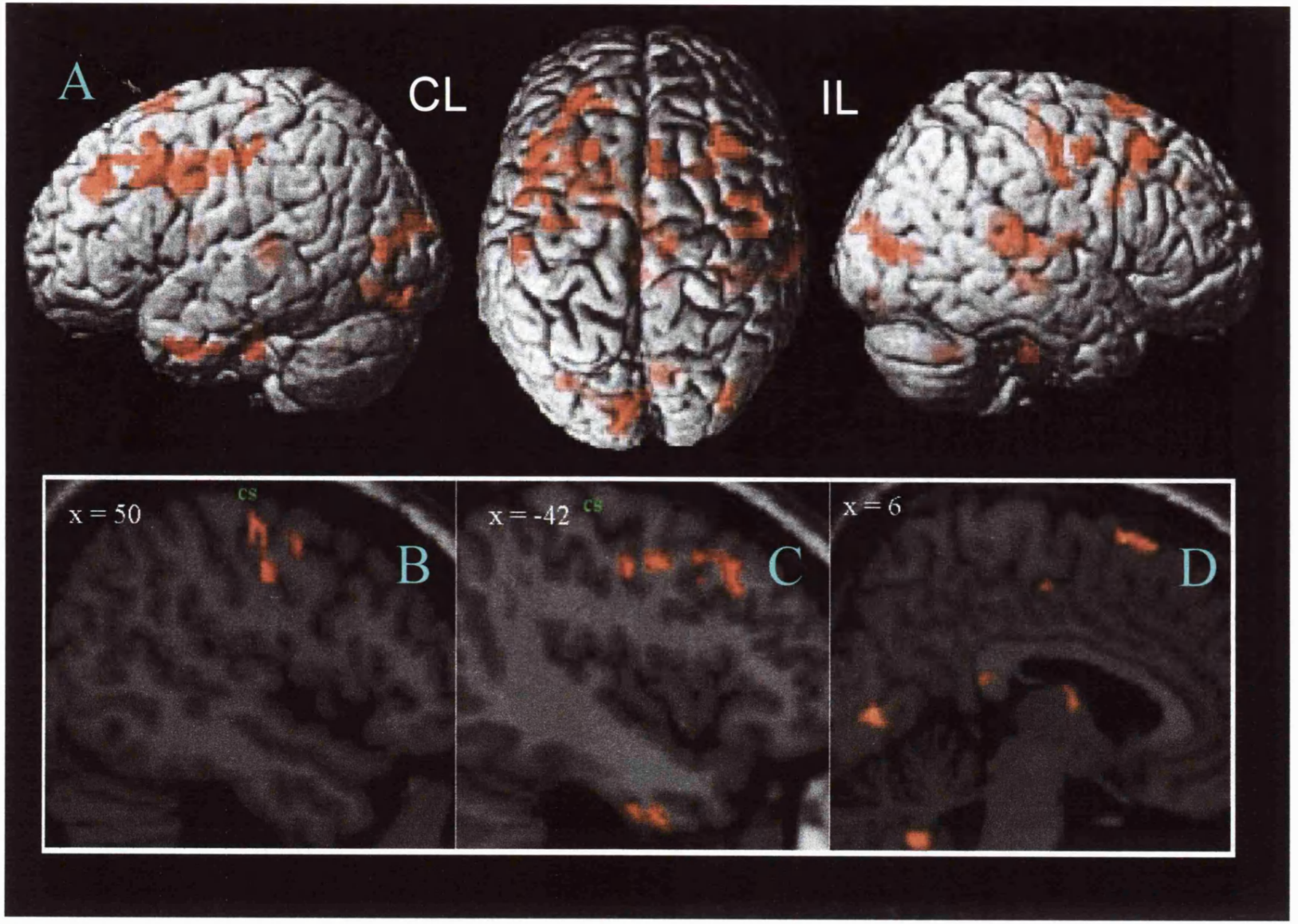


Figure 7.4

Table 7.4 *Group analysis: Regions in which activations correlate linearly with recovery*

Region	Side	Talairach coordinates in MNI space			Z-value
		x	y	z	
(a) negative correlation					
Central sulcus (M1)	I	44	-14	42	3.88
	I	52	-10	36	3.75
	I	50	-12	52	3.57
	C	-42	-10	42	3.82
PMd	I	48	-6	50	4.18
	C	-42	0	42	3.97
Anterior PMd (BA 6/8)	I	32	24	50	4.76
	C	-20	20	50	4.21
Caudal PMv	C	-46	14	38	4.05
SMA	I	8	-22	66	3.85
Pre - SMA	I	6	12	66	4.31
Cingulate Sulcus	I	4	-16	50	3.33
Prefrontal cortex	C	-24	42	32	4.01
Inferior parietal cortex (BA 40)	C	-54	-22	44	5.15*
Intraparietal sulcus	I	30	-50	50	4.23
Superior temporal gyrus	I	64	-24	8	4.19
Middle temporal gyrus	C	-50	8	-34	4.17
Striate cortex	I	6	-74	8	4.25
Middle occipital gyrus	I	36	-84	6	3.91
	C	-32	-78	4	3.84
Cerebellum	I	8	-62	-36	3.64
Thalamus	C	-2	-6	12	4.19
Putamen/globus pallidum	I	24	-8	12	4.08
(b) positive correlation *no significant voxels*					

Group analysis demonstrating regions in which there is a correlation between recovery and task related activation for all patients. Images for patients with left sided lesions were flipped about the mid-sagittal line, so that all patients were assumed to have a lesion on the right side, with initial left hand weakness. Coordinates represent peak voxels within significant cluster ($p < 0.05$, corrected for multiple comparisons across whole brain volume).*Voxels significant at $p < 0.05$, corrected for multiple comparisons across whole brain volume. I = ipsilesional, C = contralesional, M1 = primary motor cortex, PMd = dorsolateral premotor cortex, PMv = ventrolateral premotor cortex, SMA = supplementary motor area. Roman numerals for cerebellar activations refer to cerebellar lobules (Schmahmann et al., 1999).

7.3.4 Comparison of constant effort or constant force across sessions

This comparison was made only in patients in whom the initial affected grip force was < 50% of the final affected grip force, in order to ensure significant differences between target forces used in tasks A (constant effort) and B (constant force and rate). Five patients fulfilled this criterion (table 7.2).

The direct comparison of recovery-related decreases in task-related activations across sessions for tasks A and B revealed significant changes for the comparison of task B versus task A, but not for task A versus task B. Greater recovery-related decreases across session were seen in ipsilesional sensorimotor cortex (three out of five patients, $p < 0.05$ corrected for multiple comparisons), and contralesional sensorimotor cortex (two out of five patients, $p < 0.05$ corrected for multiple comparisons), for the comparison task B versus task A. This result indicates that decreases in sensorimotor cortex activation across sessions as a function of recovery are more likely to be seen if the absolute target force is maintained across sessions despite improving function.

7.3.5 Comparison with normal subjects

None of the four control subjects showed task-related linear increases or decreases across sessions at a threshold of $p < 0.05$ (either voxel or cluster), corrected for multiple comparisons across whole brain.

7.4 Discussion

I have demonstrated for the first time that there is a clear relationship between changes in motor-related brain activation and changes in performance parameters over time in patients who have suffered from ischaemic hemiparetic stroke sparing the primary motor cortex. After stroke, there is an early and widespread recruitment of brain regions during motor performance followed by a progressive reduction in this task-related recruitment over time that correlates with recovery scores in individual patients. This process occurs primarily in motor-related regions, in particular involving bilateral sensorimotor cortices, premotor cortex, supplementary motor areas, cingulate motor areas, cerebellum, basal ganglia and thalamus, but also in parietal cortex, prefrontal cortex, and striate and extrastriate cortex. Progressive increases in task-related activation were seen in different brain regions as a function of recovery in half of the patients, but there were no consistent effects across the group. Such 'focussing' of brain activation has been reported before (Marshall *et al.*, 2000; Calautti *et al.*, 2001a) and discussed in chapter 2. More recently Feydy *et al.*, (2002), described differential evolution of motor-related activation in stroke patients depending on whether primary motor cortex (M1) was involved, but no relationship with recovery was found. These authors described four cases similar to my own (good recovery, non-M1 lesions), all of whom had early bilateral activation during a motor task followed by a focussing towards a relatively normal activation pattern over three sessions. Small *et al.*, (2002) attempted to find correlations between recovery and task related activations and found only increases in ipsilateral cerebellar activation in a group analysis of patients with better compared to poorer recovery. The *post hoc*

separation of patients into groups of good or poor recoverers and the small number of recovery tests used may have contributed to a lack of sensitivity in that study.

The results described in this chapter therefore represent the first description of a voxel-wise relationship between task related brain activation and recovery. A number of important issues deserve further discussion.

7.4.1 Patient selection

Although no stroke type was actively excluded on anatomical grounds, there are clearly sources of bias in patient selection. None of the patients had significant language or other cognitive deficit because of the need to understand the experimental instructions. Furthermore, all patients had regained at least some ability to grip with the affected hand by 10-14 days post stroke. As a result the cohort consists largely of patients with subcortical infarcts and none had infarcts involving the hand region of M1. Thus the results pertain only to such patients and are not directly applicable to those with the behavioural consequences of widespread cortical damage.

7.4.2 Controlling for effort across sessions

The use of hand grip in these experiments and the attempt to control for effort during the motor task across subjects or sessions has been discussed in chapter 4. The data from this experiment afforded the opportunity to address this question empirically. Thus, in order to compare equivalent tasks across sessions within subject, I applied these principles in task A (20% MVC on the day of scanning

across sessions). That this strategy succeeded in controlling for effort across sessions is supported by the results of the VAS effort ratings completed by each subject at each session for task A. In task B however, the absolute force and rate were maintained across sessions, similar to the designs of previous longitudinal motor studies in stroke patients (Calautti *et al.*, 2001; Feydy *et al.*, 2002; Small *et al.*, 2002). The direct comparison of recovery-related decreases in brain activation for tasks A and B suggests that if absolute performance parameters are maintained and the task becomes easier over sessions, there is an increased likelihood of observing recovery-related decreases in ipsilesional and to a lesser extent contralesional sensorimotor cortex with time. This finding is consistent with the observation that difficult motor tasks result in greater recruitment within the motor system than simple motor tasks (Catalan *et al.* 1998). Thus the results with task B could be explained by a decrease in task difficulty or by neuronal reorganisation; it is not possible to differentiate between the two mechanisms *post hoc* (Price and Friston, 1999). The results for task A however, are not confounded by decreasing effort over time.

7.4.3 Early post acute changes

When comparing post-acute and chronic phase task-related brain activations, my data and those of others clearly demonstrate greater and more widespread brain activation in early compared to late stages (Marshall *et al.*, 2000; Calautti *et al.*, 2001; Feydy *et al.*, 2002). Findings in animal models of focal cerebral infarction may account for such observations. For example, an increase in both dendritic branching (Jones and Schallert, 1992) and synaptic number (Jones *et al.*, 1996;

Stroemer *et al.,* 1995) has been noticed in both damaged and undamaged hemispheres days after a lesion. Branching may overshoot and be followed by pruning back, as seen during normal development (Kolb, 1995), which may explain some of the time-related reductions in activation size.

Perhaps more interesting is the finding of widespread areas of cortical hyperexcitability appearing days after cerebral infarction and reducing over subsequent months (Buchkremer-Ratzmann *et al.,* 1996). These changes occur in regions structurally connected to the lesion in both hemispheres as a consequence of down-regulation of the α1-GABA receptor subunit and a decrease in GABAergic inhibition (Neumann-Haefelin *et al.,* 1998). The BOLD signal measured by fMRI represents primarily input and processing within a region and not the output signal (Logothetis *et al.,* 2001) so that a state of hyperexcitability will result in increased and more diffuse BOLD signal. The functional consequence of hyperexcitability is a facilitation of activity dependent plastic change (Hagemann *et al.*, 1998). Although these phenomena have been reported in animals with cortical lesions there is evidence from human studies that subcortical damage to the corticospinal pathway has a similar effect. Enlargement of the motor output zone was observed as a consequence of degeneration of the corticospinal tract (Kew *et al.,* 1994), possibly as a result of loss of recurrent inhibition onto surrounding pyramidal cells (Ghosh and Porter, 1988). There is also evidence for hyperexcitability in the contralesional motor cortex after both cortical and subcortical stroke in humans with at least moderate recovery (upper limb motricity index score ≥ 61; (Bütefisch *et al.,* 2003). Such hyperexcitability decreases with time after infarction, in keeping with data from animals (Witte,

1998; Shimizu *et al.*, 2002). Whether these changes result in increased and more diffuse task-related BOLD signal in the early human post-stroke phase, as one would predict, and whether they subserve the recovery process requires further investigation.

7.4.4 Longitudinal changes

If recruitment of independent parallel non-primary motor loops is the consequence of impairment to direct M1 cortico-motoneuronal pathways then the most parsimonious explanation for a reduction of such recruitment is that recovery of motor function is a direct result of restitution of this direct anatomical link. Several studies using transcranial magnetic stimulation in stroke patients have demonstrated changes in neurophysiological parameters from the affected hemisphere suggestive of improving corticospinal function (in particular motor threshold, motor evoked potential amplitude in a hand muscle, and central motor conduction time) that correlates with recovery of hand function (Heald *et al.*, 1993; Turton *et al.*, 1996; Traversa *et al.*, 1997; Pennisi *et al.*, 2002).

However, it is clear that abnormalities in neurophysiological parameters can persist in patients with complete recovery (Pennisi *et al.*, 2002) suggesting that preservation of fast, direct cortico-motoneuronal pathways is not the only means of achieving full recovery and that cerebral reorganisation may play an important role in generating motor output. A number of mechanisms may be involved in driving this reorganisation.

7.4.4.1 Changes in cortical motor representation provide alternative motor output. Recruitment of more ischaemia-resistant small diameter myelinated corticospinal fibres, such as those from premotor cortex, may compensate loss of large diameter fibres. Alternatively, changes in cortical representations may enable the lesioned brain to take advantage of considerable redundancy within the somatotopy of M1 (in that a number of combinations of pyramidal cells may produce the same movement (Sanes and Donoghue, 2000)) to generate an output to the spinal cord via an intact portion of the pyramidal tract. Shifts in the peak ipsilesional sensorimotor task-related activations in post stroke patients have been observed in previous studies (Weiller *et al.*, 1993; Pineiro *et al.*, 2001), and I have demonstrated that additional areas of M1 have been recruited in incompletely recovered patients (chapter 6). In a *post hoc* analysis, shifts in the peak sensorimotor activation from the first session to the last in 6 of my patients (all except patients 5 and 6) was observed, but in no consistent direction, nor seemingly correlated with the lesion site. In the group analysis I observed recovery-related reductions in activation in M1 ventral to the hand area (z = 52), and deep in central sulcus corresponding to area 4p (z = 42 and 36). I speculate that early changes such as hyperexcitability may increase the amount of M1 that is activated, facilitating subsequent refocusing towards a shifted sensorimotor representation with access to undamaged fast cortico-motoneuronal pathways in those with better recovery. In those with poorer recovery, recruitment of additional M1 regions may persist, as I have previously observed (chapter 6). Such a mechanism could not occur with damage to the entire M1 cortical region. In this respect, patients such as ours with preserved M1 are clearly different to

those with substantial M1 damage. Clinical improvement in patients with (near) complete M1 damage does occur, but is more limited and 'reorganised' motor output is likely to come from the undamaged hemisphere. Even in the patients with subcortical infarcts I observe task-related activations in contralesional M1 early after stroke that are not accountable for by mirror movements. This activation tends to diminish across sessions as a function of recovery. It is likely therefore that contralesional M1 plays some part in the recovery process, although there is no evidence that it is more important than recruitment of non-primary parallel motor networks.

7.4.4.2 Does attention to motor performance contribute to cerebral reorganisation?

Increases in task-related brain activation as a result of increased attention to a simple motor task have been observed in a number of motor regions, including SMA, cingulate cortex, insula, post-central sulcus and deep central sulcus (putative area 4p) (Johansen-Berg and Matthews, 2002; Binkofski *et al.*, 2002). It could be argued therefore that my results are largely due to the fact that early after stroke, when deficit is greatest, patients need to pay more attention to a task. I attempted to control for attention within a scanning session by incorporating visual feedback to which all subjects were asked to attend. However, reductions in both striate and extrastriate activations occurred in several patients suggesting attention to the visuomotor task was greater early on. It is possible that increased attention to a task is a key strategy early after stroke when deficit is greatest. This attentional mechanism will contribute to increased activation in non-primary motor regions, thereby providing a substrate for activity dependent plastic changes

in somatotopic representations within the motor system and consequently increasing access to alternative motor pathways. While this is not a factor I set out to manipulate experimentally, it clearly needs to be examined in future longitudinal studies.

7.4.4.3 Do stroke patients re-learn motor performance?

Current models of motor learning suggest that during early learning there is a dynamic interaction between a frontoparietal network encoding movement in terms of spatial coordinates that requires high levels of attention, and motor cortex which encodes movement in terms of a kinematic system of joints, muscles, limb trajectories *etc.* Motor cortex is dominant once learning has occurred and a movement has become automatic (Hikosaka *et al.,* 2002). Each of these cortical systems is engaged in loops that include different regions of cerebellum and basal ganglia. Attempted movements by hemiparetic patients will result in significant discrepancies between predicted and actual performance, generating error signals that in normal subjects are used by the cerebellum to optimise subsequent sensorimotor accuracy (Blakemore *et al.,* 2001). Interactions between these parallel systems occur not only in cerebellum and basal ganglia, but also via intracortical connections involving particularly premotor cortex and pre-SMA (Hikosaka *et al.,* 1999; Hikosaka *et al.,* 2002). A number of empirical findings support such a model. Decreases in brain activation as a function of motor learning have been reported in lateral premotor cortex, prefrontal cortex, pre-SMA, superior parietal cortex, anterior cingulate, cerebellum, cerebellar vermis, and caudate, (Jenkins *et al.,* 1994; Toni *et al.,* 1998; Nakamura *et al.,* 1998); the

cerebellum is involved in detecting error between internal models of movement and the sensory consequences of actual movement (Blakemore *et al.*, 2001; Imamizu *et al.*, 2000); activation in pre-SMA has been explicitly associated with new motor sequence learning (Nakamura *et al.*, 1998; Hikosaka *et al.*, 1999; Sakai *et al.*, 1999); and a variety of changes in M1, predominantly increases in recruitment, have been observed (Karni *et al.*, 1995; Grafton *et al.*, 1995; Honda *et al.*, 1998; Toni *et al.*, 1998; Muellbacher *et al.*, 2002). I describe decreases in activation with recovery in similar networks, both in individuals and across the whole group, consistent with the notion of a transfer of reliance from the frontoparietal to the primary motor system. I did not see consistent increases in activation with recovery, although they were seen in individuals. Increases and decreases in recovery-related activations in the same functional region may represent shifts in somatotopic representations. Increased activation in M1 during motor learning is predicted in the normal brain by such a model and has been observed in normal subjects when learning occurs over weeks (Karni *et al.*, 1995) but I have observed such an increase in only one patient. However, damage to direct connections between M1 and spinal cord motor neurons may prevent normal responses and result in shifts of peak M1 activation by re-mapping instead. The need to re-learn simple motor tasks after stroke is likely to engage such a mechanism, but the degree to which this can occur will depend on the degree of overall damage to the motor network. Issues such as the role of error signals generated in the damaged motor system are important and unexplored though they may have significant implications for rehabilitative interventions.

7.4.5 Conclusions

I have correlated longitudinal changes in motor-related brain activation and recovery of function in individual patients for the first time. I have been able to do this because of the large number of study sessions accompanied by detailed measurement of a variety of aspects of functional recovery in each patient. One aim of this study was to identify changes in activation patterns of relevance to the recovery of individual patients. The differences in results between patients are likely to be a result of differences in (i) anatomical damage, and (ii) cognitive parameters such as motivation, concentration and attention. I have attempted to control for the latter in order to make inferences about the former, but clearly studies from more patients are required to be able to draw firm conclusions about all recovering stroke patients. However, within my patient group I have observed some remarkably consistent patterns in recovery-related changes in brain activation patterns, which are independent of the initial site, severity and rate of recovery. My data suggest that in stroke patients with infarcts not involving primary motor cortex, there is a clear linear relationship between recovery scores and task-related brain activation in many parts of the motor system. It has been assumed that this relationship is linear, perhaps because of the lack of previous detailed studies. However, this may not be the case given that different mechanisms may facilitate recovery at different time points after stroke. Nevertheless my analysis shows that the assumption of linearity is a robust first-pass approximation.

Chapter 8

NEURAL CORRELATES OF INITIAL SEVERITY AFTER STROKE:

A CROSS-SECTIONAL FMRI STUDY

8.1 Introduction

The results described in chapter 6 clearly demonstrate that the size and distribution of task related brain activations in chronic stroke patients is related to outcome. Patients with poorer outcome recruit more of the motor system than patients with better outcome. There are no studies however that have examined whether this relationship holds outside the chronic phase of stroke recovery. I was interested to use the data acquired in the previous chapter to see whether task related brain activations in post acute stroke patients are related to the degree of initial impairment, and then to compare these results to the chronic phase correlation analysis in a single statistical test.

8.2 Materials and methods

The eight stroke patients, and experimental paradigm are described in section 7.2.

8.2.1 Behavioural evaluation

Patients were assessed with nine different outcome scores as described previously. A principal component analysis was performed on these scores to obtain an overall score of initial severity for each patient, such that the initial severity of one patient could be considered relative to any other.

8.2.2 Image analysis

8.2.1.1 Post acute phase correlation analysis

A multi-subject fixed effects model was employed. For each of the eight patients, the first session (i.e. 10-14 days post stroke) was used. All handgrips (task A and task B) were defined as a single event type, and modelled as delta functions (Friston *et al.*, 1998) using a set of orthogonalised polynomial expansions up to the third order as described in chapter 5. I examined for voxels in which there is a linear correlation between the initial severity scores and the parameter estimates for the covariates representing the main effects of hand grip across patients. The specific contrasts were weighted according to the mean corrected initial severity scores (either positively or negatively) appropriate for each patient, in order to generate an appropriate SPM{t} representing brain regions in which task-related activations correlate (either positively or negatively) with the initial severity across patients.

The resulting SPM{t}s were thresholded at $p < 0.05$, corrected for multiple comparisons across whole brain. For significant voxels, the correlation coefficient for the plot of parameter estimate against recovery for each subject, together with the corresponding *p*-value, was also calculated.

8.2.2.2 Comparison of post acute phase and chronic phase correlation analyses

The next step was to compare the correlation analysis in chronic stroke patients to that in post acute patients by direct statistical testing. This was performed in two steps. In the first stage, all single patient data were reanalysed so that the same design matrix was used for each patient. Only the first 116 image volumes were used for each patient. Using a single subject fixed effects model, all handgrips were defined as a single event type, and modelled as delta functions (Friston *et al.*, 1998) using a set of orthogonalised polynomial expansions up to the third order as described in chapter 5. The data for the second stage of analysis comprised the pooled parameter estimates for the main effects of hand grip across all subjects. Contrast images for each subject were entered into the model together with a single measure of relative recovery/outcome for each patient. The resulting design matrix is illustrated in figure 8.1. The analyses of interest comprised the direct comparison of columns 3 and 4 (i.e contrast [0 0 –1 1 0], and [0 0 1 –1 0] in two separate t-tests. Column 3 represents the normalised recovery scores for each patient in the post acute group, and column 4 represents the equivalent data for the chronic group. The resulting SPM{t}s were thresholded at $p < 0.05$, corrected for multiple comparisons across whole brain. This approach is able to identify voxels

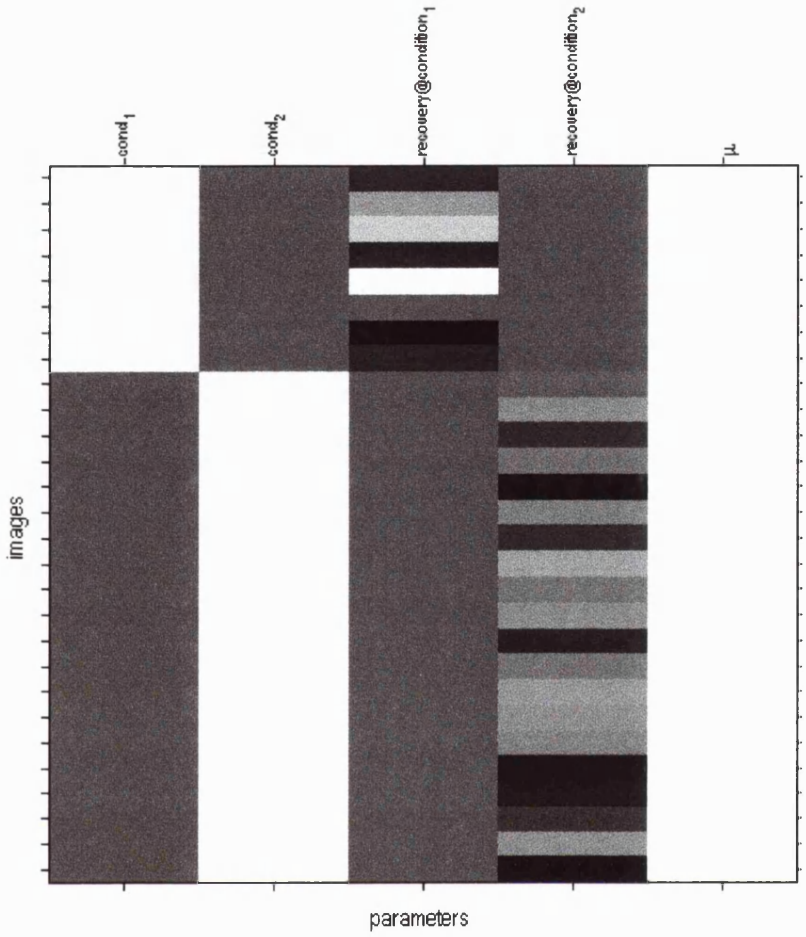


Figure 8.1 Design matrix used to compare the post-acute and chronic state correlation analyses. Condition 1 = the main effects of hand grip for post-acute patients. Condition 2 = the main effects of hand grip for chronic patients. The analyses of interest comprised the direct comparison of columns 3 and 4 (i.e contrast [0 0 –1 1 0], and [0 0 1 –1 0] in two separate one tailed t-tests. This approach is able to identify voxels for which the correlation slopes in condition 1 and condition 2 differ significantly.

Table 8.1 *Range of outcome scores*

Outcome Score	Post-acute scores			Chronic scores		
	min	max	mean (sd)	min	max	mean (sd)
Barthel	11	19	15.5 (3.6)	9	20	17.9 (3.8)
Rankin	4	2	3.1 (1.0)	4	1	2.1 (1.2)
Orpington Prognostic Scale	3.6	2	2.6 (0.6)	4	1.6	2.2 (0.7)
Action Research Arm Test	13	56	43.6 (16.7)	10	57	43.6 (18.2)
Grip Strength (% unaffected side)	3.2	91.8	55.6 (30.2)	17.8	106.9	73.1 (27.8)
Motricity Index - upper limb	62	93	70.0 (30.1)	62	100	83.4 (18.1)
Motricity Index - lower limb	40	92	77.2 (18.8)	59	100	87.2 (18.4)
9 Hole Peg Test (% unaffected side)	0	90.3	34.8 (33.2)	0	107.3	54.0 (38.2)
10 metre walk (m/s)	0	1.95	0.48 (0.69)	0	2.3	0.93 (0.64)

for which the correlation slopes in condition 1 and condition 2 differ significantly. The coefficients of correlation between task-related brain activation and recovery/outcome for each group were also calculated for significant voxels in each t-test.

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).

8.3 Results

8.3.1 Outcome scores

The early outcome scores for all eight patients are shown in table 7.2. The first principal component of all outcome scores performed at the first scanning session accounted for 77.7% of the total variance. The first principal component of all outcome scores performed at the chronic session accounted for 81.2% of the total variance.

The range of impairment and disability was similar across the two groups (table 8.1).

8.3.2 Correlation with outcome in post-acute stroke patients

A significant negative linear correlation between task-related activation and early performance levels was observed in several regions (table 8.2). Thus in the post acute phase, patients with more severe strokes are more likely to activate a number of brain regions during the hand grip task. These include bilateral M1,

Table 8.2 *Correlation between early outcome scores and task related brain activation 10-14 days after stroke*

Region	Side	Talairach coordinates in MNI space			Z-value	Correlation analysis	
		x	y	z		r^2	p-value
(a) negative correlation							
Central sulcus (M1)	I	36	-26	64	> 8.0	0.66	0.01
	C	-34	-26	70	6.91	0.49	0.05
postcentral gyrus	C	-48	-16	54	6.14	0.51	0.04
postcentral sulcus	I	32	-36	48	7.81	0.58	0.03
	C	-36	-34	52	7.53	0.8	<0.01
	C	-48	-22	48	6.14	0.8	<0.01
PMd	I	32	0	60	> 8.0	0.58	0.03
	I	32	-16	68	> 8.0	0.67	0.01
	C	-30	0	62	7.66	0.58	0.03
	C	-26	-14	68	7.24	0.49	0.05
anterior PMd (BA 6/8)	I	44	22	42	5.84	0.73	0.01
SMA	C	-2	-4	58	>8.0	0.65	0.02
Pre-SMA	C	-4	28	56	7.14	0.52	0.04
Rostral cingulate sulcus	C	-2	10	38	> 8.0	0.58	0.03
Superior parietal cortex	C	-28	-68	56	> 8.0	0.73	0.01
Intraparietal sulcus	C	-28	-72	32	> 8.0	0.56	0.03
Parietal operculum (SII)	I	64	-16	18	6.36	0.62	0.02
Prefrontal cortex (BA 10)	I	34	60	-4	7.03	0.54	0.04
inferior frontal sulcus	C	-42	46	-4	6.08	0.65	0.02
Superior temporal sulcus	I	58	-48	14	6.23	0.66	0.01
	C	-50	-44	10	6.16	0.69	0.01
Middle occipital gyrus	C	-50	-64	-4	> 8.0	0.85	<0.01
Striate cortex	I	2	-84	14	5.67	0.51	0.05
	C	-4	-96	14	> 8.0	0.56	0.03
Cerebellum - VI	C	-30	-76	-18	> 8.0	0.64	0.02
	I	22	-62	-18	> 8.0	0.64	0.02
Cerebellum - V	C	-24	-30	-34	5.59	0.66	0.01
Cerebellum - CrI	I	48	-60	-28	> 8.0	0.69	0.01
Cerebellar Vermis - VIIIB	n/a	-2	-68	-40	> 8.0	0.51	0.04
Cerebellar Vermis - VI	n/a	-4	-62	-22	6.02	0.56	0.03
Thalamus	I	2	-14	10	7.23	0.72	0.01
	C	-4	-2	6	5.25	0.59	0.02
Putamen/globus pallidum	I	26	-16	8	5.66	0.52	0.04
(b) positive correlation							
Superior temporal sulcus	I	48	18	-30	7.22	0.6	0.02
Inferior parietal	C	-50	-70	36	6.36	0.6	0.02
Caudate	C	-10	8	18	5.48	0.62	0.02

Legend for table 8.2 Images for patients with left sided lesions were flipped about the mid-sagittal line so that all patients were assumed to have a lesion on the right side, with initial left hand weakness. Voxels significant at $p < 0.05$, corrected for multiple comparisons across whole brain volume. The correlation coefficient (r^2) and corresponding p-value for the correlation analysis are also given. I = ipsilesional, C = contralesional, M1 = primary motor cortex, PMd = dorsolateral premotor cortex, SMA = supplementary motor area, BA = Brodmann area

Roman numerals for cerebellar activations refer to cerebellar lobules (Schmahmann *et al.*, 1999).

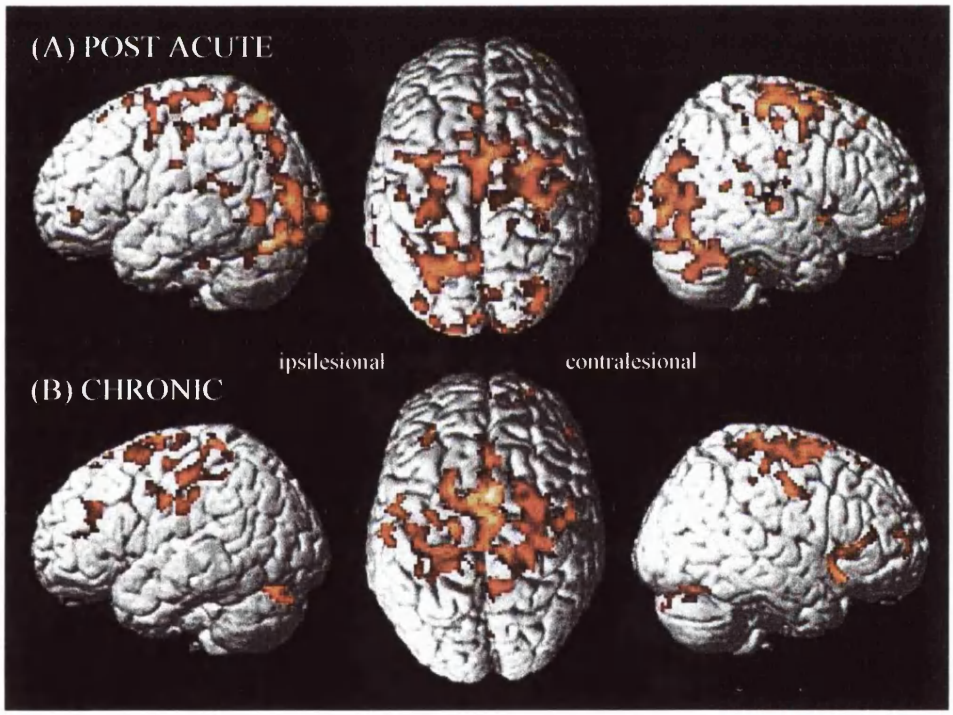


Figure 8.2 SPM{t}s representing regions in which there is a negative linear correlation between recovery and task related BOLD signal in (A) post acute patient group, and (B) chronic patient group (from chapter 6). Results are surface rendered onto a canonical brain. The brain is shown (from left to right) from the left side, from above (left hemisphere on the left), and from the right. All clusters are significant at $p < 0.05$, corrected for multiple comparisons across whole brain.

postcentral sulcus, premotor cortex, superior temporal sulcus, and cerebellum, contralesional post central gyrus, SMA, pre-SMA, rostral cingulate sulcus, parietal cortex, and cerebellar vermis. In addition this negative correlation was seen in bilateral striate cortex and contralesional middle occipital lobe (close to area V5), and also in the deep structures bilateral thalamus and ipsilesional globus pallidus.

A significant positive correlation between task-related activation and early performance levels was observed in ipsilesional anterior superior temporal sulcus, contralesional inferior parietal cortex and head of caudate (table 8.2).

8.3.3 Direct comparison of the correlation with outcome in post-acute stroke and chronic stroke patients

Given the results described in chapter 6, and in the section above, I was looking primarily for differences in *negative* correlations (figure 8.2). There were no regions in which the negative correlation between task-related brain activation and outcome/recovery was greater in chronic stroke compared to post acute stroke patients. There were however a number of regions in which the correlation between outcome scores and task related brain activation was more negative in the post acute group compared to the chronic group (table 8.3 and figure 8.3). Intraparietal sulcus (IPS) was involved bilaterally; firstly, a contralesional cluster involving both middle and posterior IPS, and secondly a small ipsilesional region deep in posterior intraparietal sulcus. This analysis was also significant in contralesional superior cerebellum (crus I), ipsilesional dorsolateral premotor

cortex and a cluster involving contralesional middle occipital gyrus and posterior superior temporal sulcus.

These results were obtained assuming that all patients had right sided lesions with left hand weakness (*i.e.* images from patients with left sided lesions were flipped about the mid-sagittal line). The reason for this is that many parts of the motor system are lateralised with reference to the hand being used. However, left and right IPS have different functions in the normal human brain irrespective of the hand used (Rushworth *et al.*, 2001). Thus it may be more important to consider left and right systems, rather than ipsilateral and contralateral systems. In view of the result described above, I carried out a post-hoc analysis using the model shown in figure 8.1, but using entirely unflipped images. The results in middle occipital gyrus, cerebellum and premotor cortex were not replicated. However, the results in bilateral middle and posterior IPS remained significant (figure 8.5). This result suggests that the finding in IPS is truly bilateral.

Table 8.3 ***Direct comparison of the correlation with outcome in post-acute stroke and chronic stroke patients***

Region	Side	Talairach coordinates in MNI space			Z-value	Post-acute Correlation Analysis		Chronic Correlation Analysis	
		x	y	z		r	p-value	r	p-value
intraparietal sulcus	C	-36	-64	50	5.99*	-0.96	<0.01	0.35	ns
	I	26	-86	14	4.93*	-0.95	<0.01	-0.26	ns
middle occipital gyrus	C	-34	-86	12	4.73†	-0.89	<0.01	0.33	ns
premotor cortex	I	34	-4	62	4.71†	-0.92	<0.01	-0.57	<0.01
cerebellum (CrI)	C	-34	-74	-20	5.02*	-0.94	<0.01	-0.54	0.01

Regions in which the correlation between outcome scores and task related brain activation was more negative in the post acute group compared to the chronic group. * Voxels significant at $p < 0.05$, corrected for multiple comparisons across whole brain volume. †Cluster significant at $p < 0.05$, corrected for multiple comparisons across whole brain volume. The correlation coefficient and corresponding p-value for early and late results are given for each region.

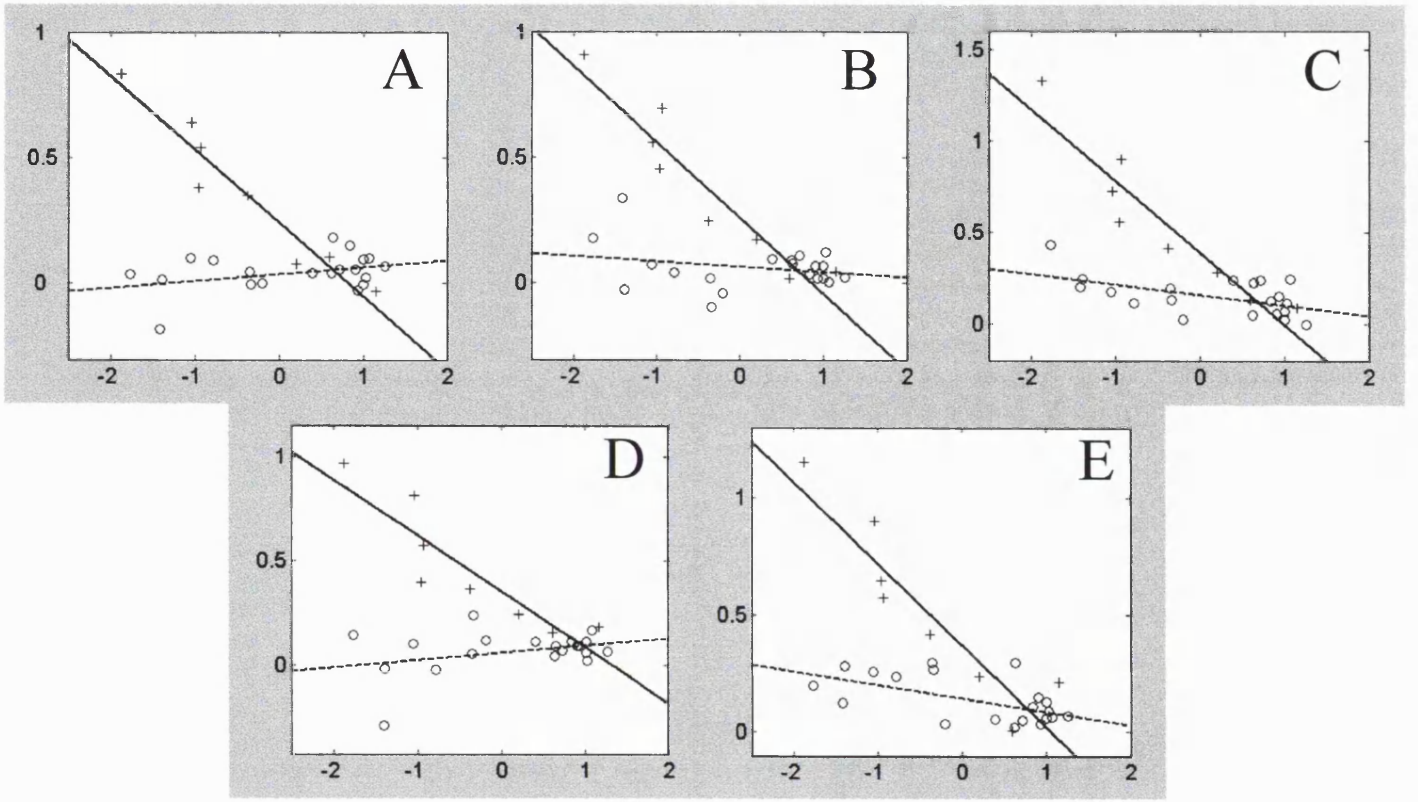


Figure 8.3 Plots of parameter estimates (task related signal change) against relative (normalised) outcome/recovery scores. Each '+' represents one patient in the post-acute group, and each 'o' represents one patient in the chronic group. (A) Contralesional intraparietal sulcus, (B) ipsilesional intraparietal sulcus, (C) contralesional cerebellum (crus I), (D) contralesional middle occipital gyrus, (E) ipsilesional dorsolateral premotor cortex. The exact coordinates and correlation coefficients are given in table 8.3.

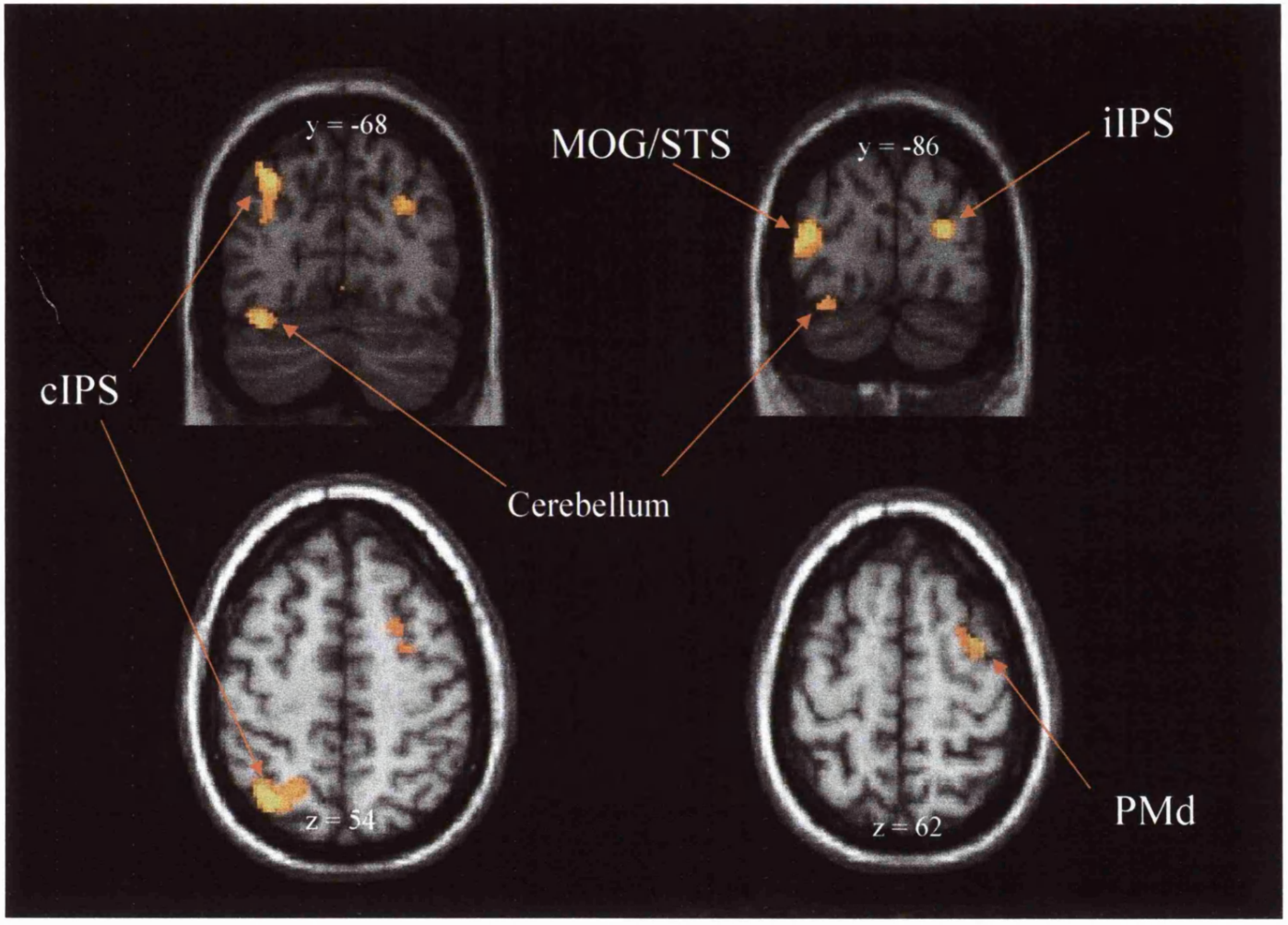


Figure 8.4 Regions in which the correlation between outcome scores and task related brain activation was more negative in the post acute group compared to the chronic group. Results displayed on canonical brain slices at $p < 0.001$, uncorrected for multiple comparisons for display purposes. The right side of brain is displayed on the right. cIPS = contralesional intraparietal sulcus, iIPS = ipsilesional intraparietal sulcus, MOG/STS = contralesional middle occipital gyrus/superior temporal sulcus, PMd = ipsilesional dorsolateral premotor cortex. The exact coordinates and correlation coefficients are given in table 8.3.

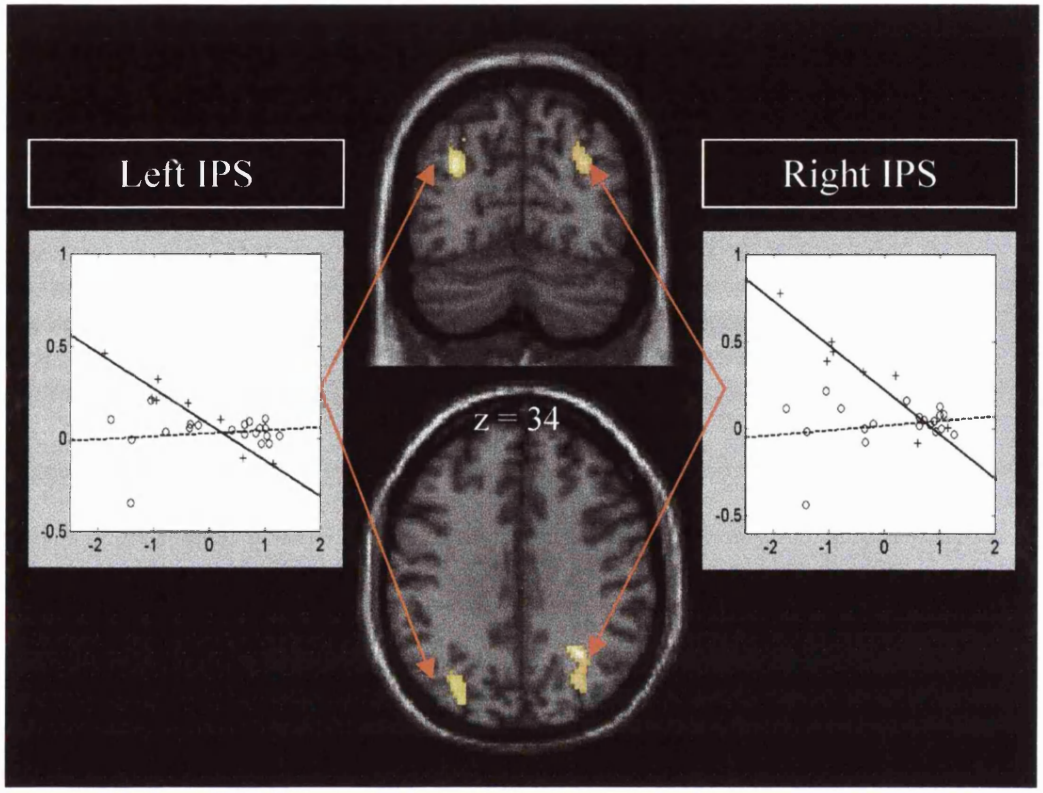


Figure 8.5 Bilateral middle/posterior IPS, in which the correlation between outcome scores and task related brain activation was more negative in the post acute group compared to the chronic group, irrespective of which hand was used in the task. Results displayed on canonical brain slices at $p < 0.001$, uncorrected for multiple comparisons for display purposes. The right side of brain is displayed on the right.

Peak voxel in left IPS (x = -30, y = -72, z = 28, Z-score = 4.38). Peak voxel in right IPS (x = 32, y = -70, z = 30, Z-score 4.15).

Plots of parameter estimates (task related signal change) against relative (normalised) outcome/recovery scores for each peak voxel are shown. Correlation coefficients for left IPS (Post- acute: $r = -0.96$, $p < 0.01$. Chronic: $r = 0.14$, p = ns). Correlation coefficients for right IPS (Post- acute: $r = -0.93$, $p < 0.01$. Chronic: $r = 0.21$, p = ns).

8.4 Discussion

I have previously demonstrated overactivations in chronic stroke patients compared to normal subjects, particularly in those with poorer recovery (chapter 6). Furthermore I demonstrated a negative correlation between outcome and task related signal change in a network of primary and non-primary motor regions. I have suggested that stroke patients rely on independent parallel motor loops (Strick, 1988) in proportion to the degree of damage to the direct projection from M1 to spinal motor neurons. Parallel motor loops are useful in as much as they provide alternative input to the spinal cord motor neurons (Maier *et al.*, 2002).

I have obtained a similar result by performing a correlation analysis across a group of patients 10-14 days post stroke. In other words, patients with greater initial deficit recruit more widely and to a greater extent in a number of primary and non-primary motor regions than those with lesser deficit. My results in post acute patients suggest that the brain regions comprising the parallel motor loops are available to participate in the generation of a motor act very early, possibly immediately after stroke, rather than slowly being recruited over time.

It is of interest to consider whether different ways of generating motor output are employed at different stages after a stroke. However, the direct comparison of average brain activation patterns at early and late stages after stroke would be confounded by the fact that each group would contain patients at different stages of recovery. This is a crucial consideration because I have demonstrated that there is a correlation between degree of recovery and the pattern of brain activation in both post-acute and chronic stroke patients. The direct statistical comparison of the correlation analysis from each group is a novel way to overcome this problem.

This analysis demonstrated a stronger negative correlation between task related activation and degree of recovery in the post acute phase compared to the chronic phase in bilateral middle and posterior IPS, contralesional superior cerebellum (crus I), ipsilesional dorsolateral premotor cortex and a cluster involving contralesional middle occipital gyrus and posterior superior temporal sulcus.

My *a priori* approach to the analysis was to flip the brains of those with left sided lesions, so as to consider regional lateralisation with respect to the hand being used. This approach is often used when studying the motor system of stroke patients with different lesion locations. It is an appropriate approximation when considering regions such as premotor cortex and cerebellum. However, there is evidence that systems employed in higher cognitive aspects of motor behaviour such as motor attention may be lateralised independent of the hand used (Rushworth *et* al., 2001). I therefore performed a *post hoc* analysis without flipping brain images. Results from this analysis are considered in terms of left or right rather than contralesional or ipsilesional hemisphere. This procedure confirmed that the difference in correlation analysis is seen in middle and posterior IPS bilaterally, irrespective of hand use. The other regions were not significant in this *post hoc* analysis, indicating that location of significant regions can be considered in terms of contralesional or ipsilesional hemisphere, rather than left or right hemisphere.

Post-acute but not chronic patients with the most deficit engaged bilateral IPS during the visuomotor task. Anterior IPS is certainly activated in normal subjects during hand grip (chapter 5). Indeed, it has been implicated in a human 'grasping circuit' (Jeannerod *et al.*, 1995). However, it was middle and posterior IPS that

were most used by those with an initial severe deficit. Posterior parts of IPS have been implicated in attention during visuomotor tasks (Nobre *et al.*, 1997, 2000; Gitelman *et al.*, 1999). More recently Shikata *et al.*, (2003) suggested that it is the middle part of IPS that is related to visuomotor attention. In my study it is not possible to differentiate functional roles for middle or posterior IPS, as the significantly activated clusters span both regions.

The finding of increased contralesional middle occipital gyrus/superior temporal gyrus activation in post acute patients with greater deficit is in keeping with the idea of increased recourse to networks involved in attentionally demanding visuomotor tasks. In addition, attentionally demanding tasks are known to increase activation in a number of motor related structures, including rostral dorsolateral premotor cortex (Simon *et al.*, 2002) and superior cerebellum (Allen *et al.*, 1997).

It is tempting to speculate that the increased attention to task augments activation in ipsilesional premotor cortex, which enables the generation of additional motor signals to spinal cord motor neurons. As I have discussed however, parallel motor loops are unlikely to be able to fully substitute for fast direct projections from M1 to spinal cord motor neurons. In the patients with greatest deficit, the motor task is not only likely to be the most attentionally demanding, but is likely to generate the most error signal. Such error signal is detected in the superior cerebellum (Blakemore *et al.*, 2001), very close to the region reported in this chapter.

Thus a plausible explanation of these results is that the motor task is more attentionally demanding for those stroke patients with greatest deficit, but only in the post-acute phase. It is possible that modulation of attention is no longer useful to chronic patients in optimising performance. It is also possible that error signal

is not generated in the same way as in the post acute phase, because the degree of sensory feedback is now closer to that 'expected'.

These results are of interest. The suggestion is that different mechanisms are available to maintain performance in different stages of recovery. This conclusion has clear implications for rehabilitative techniques, and in particular for the use of attentional modulation as a therapeutic tool.

Chapter 9

GENERAL CONCLUSIONS

9.1 Introduction

In this chapter I will summarise the main results arising from the experiments described in this thesis, and will formulate an overview of the processes of cerebral reorganisation that follow focal brain injury due to ischaemia. I will then discuss the key methodological issues that need to be considered by future investigators in this field. Lastly, I will speculate on future experimental questions that might be explored.

9.2 Summary of results

9.2.1 Recruitment of non-primary motor networks

In chapter 6, I demonstrated that chronic stroke patients activate primary and non-primary motor regions over and above normal subjects, as demonstrated in many previous studies (see chapter 2). My results extend these previous findings by showing that patients who do overactivate tend to be those with poorer outcome. More formally I demonstrate a negative linear correlation between outcome and

task-related activation in a number of motor related regions. I have interpreted these results in relation to the *likely* degree of damage to the direct pathway from primary motor cortex to motor neurons in the spinal cord. Patients with severe disruption to this pathway will suffer from greater clinical deficit. If the direct input to spinal cord motor neurons is diminished, there will be a need to increase the motor signals to spinal cord motor neurons *via* alternative pathways in order to generate an output to the musculature. The work of Strick (1988) and others suggests that independent parallel loops involving premotor (both lateral and medial wall), parietal and subcortical regions exist in primates. These parallel systems provide an ideal substrate for such alternative pathways and are likely to contribute to recovery. However, the projections from premotor cortices are less numerous and efficacious than those from primary motor cortex (Maier *et al.*, 2002). This explains why bilateral recruitment of such regions does not result in full recovery of hand function.

In chapter 8, I demonstrate that the relationship between task-related activations and clinical deficit holds true at 10-14 days post stroke. This finding supports the idea that non-primary cortical motor regions are recruited as a function of the degree of damage to fast cortico-motoneuronal pathways. It also indicates that they are available to participate in the generation of a motor act very early after stroke, rather than being slowly recruited over time. There are however important differences in the functional anatomy of the visuomotor hand grip task at early and late time points after stroke. Greater task related activation is seen in brain regions that are implicated in attention to visuomotor tasks in the post-acute compared to the chronic phase. This activation is seen mainly in those with greater

initial deficit. This result provides evidence that attention is important in those with greater deficit, but also that attention may play a lesser role in the chronic phase. However, I have not explicitly tested this specific hypothesis. The role of attention deserves further investigation as it has clear implications for the timing and design of rehabilitation techniques.

In chapter 7 I describe the results of correlation analyses (between task related signal change and recovery scores) *within* subjects over time, rather than *across* subjects. The results are predictably different in each individual, but there are longitudinal changes that are common across subjects in a number of brain regions. These changes closely resemble those seen in motor learning experiments in normal individuals and are similar to those predictable on the basis of contemporary models of motor learning.

I have hypothesized that damage to cerebral structures necessitates the engagement of alternative pathways, but that these pathways were previously not necessary and therefore were dormant. There is one piece of evidence that suggests that some regions are not only recruited over and above normal patterns, but that they function in a different way. In chapter 5, I describe how activation may change linearly or non-linearly in relation to increasing hand grip force in normal subjects. In chapter 6 I demonstrate in chronic stroke patients that linear increases in activation are found with increasing grip force in deep central sulcus (BA 4p), postcentral gyrus, premotor cortex, and bilateral anterior intraparietal sulci, but more so in patients with poorer recovery. In other words, this part of the motor system is not only increasingly recruited in patients with poorer outcomes, but it also *behaves* in a different way during task performance. It is as if these

regions have taken on an executive function, as if reprogrammed. This finding requires further exploration, as it may provide compelling evidence of true lesion induced reorganisation in the human central nervous system.

9.2.2 Recruitment of ipsilesional primary motor cortex

In chapter 6 I also report a negative correlation between outcome and task related activity in two parts of ipsilesional primary motor cortex (M1). The first is in area 4p and the second ventral to the hand region. Only the more ventral region is beyond that activated by the control group, suggesting a ventral shift in task related M1 activation in some patients with poorer outcome. In the longitudinal analysis consistent recovery-related reductions in activation are observed in M1 ventral to the hand area and deep in central sulcus corresponding to area 4p. The changes in ipsilesional M1 representation demonstrated in the cross-sectional and longitudinal studies mirror one another to a remarkable degree. Increased attention to the task might lead to increased recruitment of area 4p (Johansen-Berg & Matthews, 2002; Binkofski *et al.*, 2002). Alternatively, early lesion induced hyperexcitability may increase the extent of M1 that can be activated. Either way this process could facilitate re-mapping towards a shifted sensorimotor representation, one with access to undamaged fast cortico-motoneuronal pathways. Such a mechanism could clearly lead to improved motor performance. Failure to remap to 'connected' M1 however will lead to persistent recruitment of additional M1 regions, with consequent poorer recovery.

Plastic changes in somatotopic representations may occur not only in M1, but also in supplementary motor area (SMA), cingulate motor areas (CMA), and premotor

regions. This idea is supported by my results. Parts of SMA, CMA and premotor cortex were recruited by chronic patients with poorer outcome (chapter 6), or by patients in the early compared to late stages of recovery (chapter 7). These activations were often outside regions normally activated by the task in the control group. Such reorganisation could result in (i) stronger connections with different regions of M1 (i.e. with alternative representations of the hand region) in order to generate an output to the spinal cord; and (ii) recruitment of more surviving ischaemia-resistant small diameter myelinated corticospinal fibres, such as those arising from premotor cortex, to compensate for loss of large diameter fibres.

9.2.3 Recruitment of contralesional primary motor cortex

The role of ipsilateral M1 in the generation of normal hand movements remains controversial, but its potential role in recovery of motor function after stroke has always generated much interest. In chapter 5, I demonstrate a relative deactivation in ipsilateral M1 during hand grip in normal subjects. However, this deactivation became less pronounced with increasing age. Transcallosal inhibition is thought to reduce cortical excitability in ipsilateral M1 and decreased cortical excitability manifests as decreased BOLD signal (Hamzei *et al.*, 2002). Thus the finding of a lesser reduction in ipsilateral M1 BOLD in older compared to younger subjects can be interpreted as evidence for reduced transcallosal inhibition.

In both the cross-sectional and longitudinal stroke studies a negative correlation between task-related activity and recovery is observed in contralesional (ipsilateral) M1. However, this is not in BA 4a, but deep in central sulcus corresponding to BA 4p. Furthermore, although chronic patients with good

recovery show a relative deactivation in ipsilateral BA 4p, those with poor recovery do not merely have reduced deactivation, as do elderly controls, they had task related *activations*. Rather than representing an epiphenomenon, it is more likely that ipsilateral 4p is used for the motor task by these patients. Thus it remains plausible that parts of contralesional M1 are able to generate a motor output to the affected hand. This output could be of particular use to patients with a significant deficit in whom a dependency on alternative motor projections has developed. However there is no evidence that this process is more important for recovery than recruitment of ipsilesional non-primary parallel motor networks.

9.2.4 Overview and caveats

My results are likely to reflect a number of processes occurring after focal brain damage at both cellular and systems levels which contribute to cerebral reorganisation and functional recovery after stroke. Early on, following cerebral infarction, any voluntary movement is associated with massive recruitment of areas throughout the motor system, possibly because of alterations in the tonic reciprocal influence of anatomically connected motor-related regions upon each other. In addition a number of mechanisms described in animal models of focal cerebral ischaemia, such as local and distant cortical hyperexcitability, may play a role. Thereafter, it is likely that surviving elements of highly preserved neural systems subserving motor learning facilitate a transition from attention-dependent effortful movement to a more automated performance. This transition is accompanied by recovery-dependent shifts in the patterns of activation. The degree to which any of these elements are successfully employed in the recovery

process will depend on a number of other variables, not least the precise amount and site of anatomical damage caused by an infarct and the amount of retraining available to the patient.

It is worth noting that not all these processes could occur in patients with damage to the entire middle cerebral artery territory. I have speculated that the prime aim of cerebral reorganisation after focal damage to the motor system is to re-establish a connection between ipsilesional motor cortex and spinal cord motor neurons *via* fast direct cortico-motoneuronal pathways. This scenario provides the greatest chance of recovery of fractionated finger movements. I speculate that one way this is achieved is by shifts or re-mapping of somatotopic representations in M1 and in those parts of the motor system that project to M1. If however, there are no connections left due to the degree of damage, then parallel motor loops become useful only for their projections to spinal cord, not by virtue of their projections to M1. In this respect, patients such as ours with preserved M1 are clearly different from those with substantial M1 damage. Clinical improvement in patients with complete M1 damage does occur, but is more limited, and 'reorganised' motor output in those cases may come from the undamaged hemisphere. Studies in adults with damage to the entire middle cerebral artery territory are scarce. However, data from young adults who suffer unilateral brain damage in the perinatal period suggest that the ipsilateral hemisphere becomes the main source of motor output (Cao *et al.*, 1994).

One final caveat should be mentioned. After injury-induced reorganisation of the brain the capacity for subsequent adaptive change is reduced (Kolb *et al.*, 1998). I describe adaptive changes in older brains (chapter 5) and these may themselves

limit the capacity for further reorganisation after injury in older patients. This notion has implications for what can be expected from therapy designed to promote cerebral reorganisation after stroke in older subjects.

My conclusion is that cerebral reorganisation undoubtedly contributes to functional recovery after stroke, but it is clear that a more detailed understanding of the process and the factors that influence it is required before such information can be used to rationalise therapeutic strategies in individual patient groups. To that end, my results further the understanding of likely dynamic mechanisms underlying functional recovery after stroke.

9.3 A critique of the methodology

My experiments have extended our understanding of cerebral reorganisation in the brain after focal ischaemic damage. The principal reason is the inclusion of patients with different degrees of recovery and at different stages of their recovery. Thus, inferences can be made about the relationship between brain reorganisation and the recovery process. I will discuss how two crucial aspects of experimental design contributed to the ability to make such inferences. I will then discuss how recent findings concerning differential haemodynamic coupling between patients and controls impact on my results and future experimental design.

9.3.1 Recovery scores

Recovery is best characterized by a combination of a number of scores (Duncan *et al.*, 2000; Turton and Fraser, 1986). Such a combination is problematic, not least

because of the likely inclusion of scores that vary with recovery in a linear or non-linear fashion as well as scores that assess very similar and hence covarying parameters. In chapter 4, I describe an approach to combining scores using principal component analysis (PCA). This approach is advantageous because it allows a single measure of outcome or recovery for each patient to be calculated. Most importantly it allows the quantification of (i) the amount of variance in a multiple parameter data set by the first principal component; and (ii) the contribution from each of the original parameters to the first principal component. In all cases the first principal component accounted for a sizeable percentage of the total variation described by all the clinical recovery scores measured. Perhaps just as important was the finding that each test of recovery contributed roughly equal amounts to the first principal component. This indicates that each individual outcome measure is making a useful contribution to assessment of recovery. To illustrate this point, I used forward model selection to explore whether the addition of another measure of recovery would have any impact on the recovery score derived by PCA. The Ashworth scale of muscle tone was recorded for all patients, but out of ten outcome measures, its contribution towards the first principal component was only 3% (compared to 10-12% for the others). Ultimately it was not included because it is not considered a sensitive measure of tone (although there are none better). Furthermore, the relationship between changes in tone and outcome is not straightforward. Increases in tone may be of benefit in some cases and detrimental in others.

In summary, principal component analysis has proved a useful and robust method for combining a number of outcome scores.

9.3.2 Motor paradigm

Employing a task that could be performed by all patients with even very limited motor function enabled me to study patients with different degrees of recovery. Many other studies have used some form of fractionated finger movements, a task that has the immediate disadvantage of excluding patients with poorer recovery and concentrating on pyramidal function. It is tempting to think that because we are ultimately interested in fractionated finger movements, then this might be the best task to use. *However it is not possible to scan patients to determine the neural correlates of something they cannot do.* The experimenter must find an alternative way to probe the system of interest. My solution to this problem has been to use a more rudimentary motor task with sensory (in this case visual) feedback, thereby introducing elements of visuomotor control. Thus in all my experiments the comparisons are between subjects performing a repetitive hand grip task at a fixed proportion of their own maximum handgrip. This is therefore a task that they *can perform i.e.*, all patients were able to perform repetitive hand grips at 10%, 20%, 40% etc. of their own maximum. The results of the comparisons then pertain to how a damaged motor system is able to perform this task. For example, one study suggests that finger tapping is more sensitive at detecting differences in activation patterns between stroke patients and controls than a squeezing task (Cramer *et al.*, 2001). I would argue that this is because the differential effort exerted by patients compared to controls is greater for finger tapping than it is for squeezing. Because of this potential confound, the finger tapping task is *less* efficient at detecting differences of interest. Advocates of the use of fractionated finger movements as an experimental paradigm will point out that the increased effort used by patients

with incomplete recovery is important tool for recovery and hence of interest. This is correct; nevertheless such a paradigm leads to difficulties in interpretation. Comparisons across subjects can be explained by differences in cerebral reorganisation or by differences in effort or an interaction between the two. The former is explicitly the variable of interest in my experiments, necessitating attempts to control for the latter. The fatigue that stroke patients experience makes this difficult, but the results of my comparisons of visual analogue scores both across and longitudinally within subjects suggest that the attempt has been successful.

9.3.3 Haemodynamic coupling

In the experiments described in this thesis, the BOLD response has been modelled in both patients and controls using the same canonical haemodynamic response function. Recent evidence has thrown some light onto the question of whether this approach is valid.

Newton *et al.*, (2002) demonstrated a greater time to peak BOLD response in ipsilateral compared to contralateral M1 in controls. In three chronic stroke patients the time to peak BOLD response was increased in ipsilesional (contralateral) M1 compared to controls. Interestingly, in these patients the time to peak BOLD response in contralesional M1 was equivalent or less than that for ipsilesional M1, representing a finding opposite to normals. The reason for such a finding is not clear. Pineiro *et al.*, (2002) have also described a slower time to peak BOLD response in sensorimotor cortex bilaterally in 12 chronic stroke patients with lacunar infarcts. This finding suggests that the presence of small

vessel disease may be the cause, rather than the infarct itself. Thus modelling the BOLD response with a canonical haemodynamic response function might be less efficient in stroke patients. The effect would be to increase the residual error of the analytical model, thus *lowering* t- and Z-scores and depressing sensitivity to detection of differences. In fact, in my experiments I have mostly observed overactivations in patients compared to controls, so it might be the case that these overactivations have been *underestimated.* In the case of chronic patients in whom no differences could be found in task-related activation patterns compared to controls, it is possible that real differences were not detected. In these cases, the null-hypothesis cannot be rejected. In future, increased sensitivity may be gained by modelling any differences in haemodynamic responses, but failure to do this in my experiments is highly unlikely to have led to any false positive results.

Röther *et al.*, (2002) provide some interesting insights into the relationship between the BOLD signal and haemodynamic reserve. They studied a single subject with bilateral occluded internal carotid arteries and an occluded vertebral artery (side not specified). This subject was found to have severely impaired cerebrovascular reactivity in the left hemisphere, as determined by reduced change in T2*-signal (at 1.5T) during hypercapnia. The finding of importance was that during a motor task with the right hand, the BOLD response in the left motor cortex was negative for the duration of the task. In other words, the transient uncoupling between blood flow and cerebral metabolism that results in the 'negative dip' in BOLD signal (see chapter 3) was prolonged. This subject had previously suffered from a transient ischaemic attack involving the right arm. It is likely that these symptoms were related to haemodynamic insufficiency and it is

interesting to speculate that the presence of a prolonged negative dip in BOLD signal represents a marker for those at risk from such symptoms. Further investigation will reveal whether this idea has genuine potential as a clinical tool. Thus in patients with severely impaired cerebrovascular reactivity neuronal activation may not translate into a BOLD response in the conventional sense, and standard models using canonical haemodynamic response functions may not be sufficient. Having said that, the cerebrovascular reactivity in the right hemisphere of this patient was moderately impaired, and the BOLD response during a motor task with the left hand was entirely normal. Patients with haemodynamic symptoms are rare, and were excluded from my studies. However, this case provides a useful insight into the effects on the BOLD response of severely impaired cerebrovascular reactivity. More importantly from the point of view of my studies, it highlights the fact that the BOLD response is likely to be relatively robust to moderately impaired cerebrovascular reactivity. Patients with severe stenosis of ipsilesional internal carotid arteries are usually excluded from fMRI studies. This case suggests that such an approach may not be necessary, but further studies are required.

The discussion regarding differences in haemodynamic coupling is also of relevance when considering the effects of age. D'Esposito *et al.*, (1999) found little difference in the shape of the haemodynamic response in sensorimotor cortex between groups of young and old subjects. However, the median number of suprathreshold voxels was four times greater in the young compared to the older group. Therefore, the authors suggested that neuronal activation might fail to evoke a BOLD response in some brain regions in elderly subjects, *i.e.*, produce a

false negative result. In practice this means that a comparison of young and old subjects that finds less activation in young than older subjects is unlikely to be accounted for by differences in haemodynamic coupling, whatever the mechanism. The results in chapter 5 demonstrate *increased* task-related activation in parts of the motor system in older subjects and so are unlikely to be confounded by the differences described by D'Esposito *et al.*, (1999).

9.4 Future directions

My experiments have contributed to an understanding of the way the motor system responds to ischaemic injury in relation to functional recovery. Future experiments should aim to refine current concepts, with a view to providing a neurobiological rationale for neurorehabilitation. Not only would such a programme provide an empirical basis from which to investigate specific interventions in clinical trials, but it should provide a means of stratifying patients according to likely response to therapeutic interventions. Two specific areas require attention to achieve this aim.

9.4.1 Structure and function in the damaged brain

I have already suggested that the prognosis for upper limb motor recovery is a function of (i) the integrity of fast direct cortico-motoneuronal projections from M1 to spinal cord, and (ii) the functional integrity of connections between remaining cortical motor regions. Transcranial magnetic stimulation allows assessment of the integrity of cortico-motoneuronal pathways. In addition, it is now possible to quantify structural characteristics of a lesion using voxel-based

morphometry (VBM) and diffusion tensor imaging (DTI). VBM and DTI are both MRI techniques. VBM allows objective characterisation of grey and white matter differences in structural MRI scans (Ashburner & Friston, 2000, Good *et al.*, 2001), providing objective anatomical data concerning regions of the brain that have been damaged. DTI is a technique that provides quantitative information about the integrity and orientation of white matter fibre tracts in the brain (Parker *et al.*, 2001). Both will enable the construction of statistical maps of the likelihood of damage to connections between cortical areas and between cortical areas and motor output pathways in stroke patients.

These techniques can provide an objective measure of not only size and location, but the integrity of white matter connections between regions, which I hypothesize are crucial for recovery. The ability to correlate this information with task-related brain activations will provide better insight into the functional anatomy of recovery.

9.4.2 The neural correlates of therapeutically driven change

A number of studies have emerged recently that have attempted to evaluate therapeutically driven change (Johansen-Berg *et al.*, 2002b; Carey *et al.*, 2002; Schaechter *et al.*, 2002; Jang *et al.*, 2003). The methodologies of these studies have differed. In general they have shown increased task-related activation in affected hemispheres and reduced activation in unaffected hemispheres after a period of treatment. In these group analyses it is difficult to be sure what the changes reflect, particularly in view of how much more we need to know about structure–function relationships in the damaged brain. It is not clear that the

changes are 'therapeutically driven', but they may be merely a consequence of improvement of function. This would be in keeping with the focussing towards a 'normal' activation pattern seen in chapter 7. Functional imaging will not be useful purely as a marker of clinical improvement. The clinician is able observe or measure this in the tasks that the patient can or cannot perform. Functional imaging will be useful as a marker of the *potential* for change in the damaged brain. Furthermore, it is to be hoped that the potential of different therapeutic interventions can be assessed, both in groups and in individuals. It would also be of interest to determine how different patients respond (in a single session) to different experimentally controlled therapeutic manipulations. This could be used as a predictor of likely response to a particular treatment. Functional imaging, in tandem with other techniques such as transcranial magnetic stimulation, electro-encephalography, and magneto-encephalography, has the potential to achieve these aims. However, as with all experimental techniques it will require that the purpose, the design, and the analysis of the experiment be undertaken with the utmost care and thought.

APPENDIX I

DOCUMENT FOR RECORDING CLINICAL DATA AND OUTCOME MEASURES

The following document was used to record clinical details for each patient enrolled in the study. The outcome measures were recorded at each visit. The particular methods employed to record these data are detailed in the following sections.

THE NATIONAL HOSPITAL FOR NEUROLOGY AND NEUROSURGERY

Queen Square, London, W.C.1
0171-837 3611

PATIENT'S SURNAME OTHER NAMES ADDRESS Tel. No:	Admission FIRST SECOND THIRD	HOSPITAL No.	SEX M F	STATUS S M W D
		OCCUPATION		RELIG

	Date of Birth	Age	Place of Birth
	Name & Address of General Practitioner: Tel. No:		

STUDY

INFORMATION ON ADMISSION

Adm. Date		Ward	Bed Type N.H.S. Private
Consultant		SOURCE	
DISCHARGE DATE		DISCHARGED TO	

DATE OF STROKE		DATE OF ASSESSMENT	
TIME TO fMRI		TIME TO TMS	
DIAGNOSIS (1) (2)		DIAGNOSIS (3) (4)	

W 142

Clinical History

Presenting History

Risk Factors

	Yes	No	
Previous TIA/ Stroke			.....
Hypertension			.....
IHD			.....
Other cardiac disease			.....
Peripheral Vascular Disease			.....
Diabetes Mellitus			.....
Hyperlipidaemia			.....
Smoker			.....
Alcohol _____ u/week			.....
Migraine			.....
OCP			.....
Fam Hx Stroke			.....
IHD			.....
Thrombophilia			.....

Other previous medical history

1. _____
2. _____
3. _____
4. _____
5. _____

Medication

_____
_____
_____
_____
_____

Personal History

Early Development:

Schooling:

Occupation:

Pre-stroke function:

	Normal	Abnormal
Ambulation		
Bathing		
Continence		
Dressing		
Mental State		
Hearing		
Vision		

Systemic Enquiry

CVS: GU:

RS: CNS:

GI: Locomotor:

Examination

Physical Appearance:

Mental State:

Mobility:

Hand Preference:

General Examination

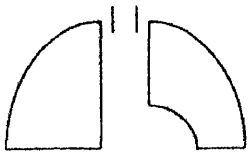
General examination:

Temp. .....°C

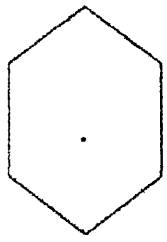
CVS: Pulse ..... bpm reg. / irreg
BP ...../..... mm Hg
JVP
HS

	Yes	No
L carotid bruit?	☐	☐
R carotid bruit?	☐	☐
Oedema?	☐	☐
Absent peripheral pulses?	☐	☐

RS:



Abdomen:



Locomotor:

Pressure Areas:

Intact ☐ Problems ☐

Neurology Examination

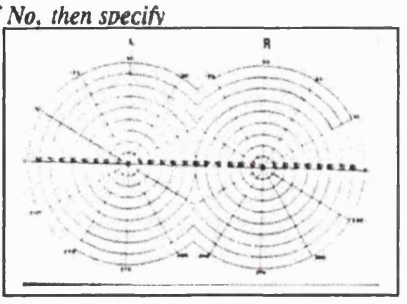
Cranial Nerves *(if not possible to test, record reason)*

		Right	Left
I			
II	fundi		
	acuity		
III/IV/VI			
	eye movements		
	pupils		
	ptosis		
	I		
V	II		
	III		
	motor		
VII	Facial palsy: none/dubious	☐	☐
	present	☐	☐
VIII *(Hearing)*			
IX / X *(Palate)*			
XI *(Sternomastoids)*			
XII *(Tongue)*			

Are the visual fields normal?
(?inattention)

☐ Yes ☐ No

If No, then specify



Swallowing Assessment

	Yes	No
Drowsy	☐	☐
Confused	☐	☐
Unable to sit up	☐	☐
Unable to cough	☐	☐
Abnormal gag	☐	☐
'Wet' voice	☐	☐
Suspected aspiration	☐	☐

If Yes to any of the above, request swallowing assessment from speech therapist and refer to dietitian.

Reflexes

	R	L		R	L
Jaw. J.			Abd. Upper		
Biceps			Abd. Lower		
Supinator			Knee		
Triceps			Ankle		
Finger			Plantar		

Motor Examination

Tone: Modified Ashworth Scale

0 No increase in muscle tone
1 Slight increase in muscle tone, manifested by catch and release, or by minimal resistance at the end of motion when the affected part is moved in flexion or extension
2 Slight increase in muscle tone, manifested by catch, followed by minimal resistance throughout the remainder (less than half) of the remainder of movement
3 More marked increase in muscle tone through most of ROM, but affected parts move easily.
4 Considerable increase in muscle tone, passive movement difficult
5 Affected part rigid in flexion or extension.

LUL _____ RUL _____

LLL _____ RLL _____

Comments _____

Limb Power:

	L	R		L	R
Shoulder Abduction			Hip Flexion		
Shoulder Adduction			Hip Extension		
Elbow Flexion			Knee Flexion		
Elbow Extension			Knee Extension		
Wrist Flexion			Ankle Dorsiflexion		
Wrist Extension			Ankle Plantarflexion		
Finger Flexion			EHL		
Finger Extension					
1st DIO					
APB					

Motricity Index

1. Pinch grip (see ARAT)
2. Elbow Flexion
3. Shoulder Abduction
4. Ankle Dorsiflexion
5. Knee Extension
6. Hip Flexion

0 - no movement
9 - No movement, but palpable contraction
14 – Movement, but not full range or against gravity
19 – Movement, full range, but not against resistance
25 – Movement against resistance, but weak
33 – Normal power

OPS

Lying supine, patient flexes shoulder to 90°

0.0	normal power	(grade 5)
0.4	reduced power	(grade 4)
0.8	movement against gravity	(grade 3)
1.2	trace movement	(grade1-2)
1.6	no movement	(grade 0)

Describe flexor/ extensor synergies and any associated movements

_____

_____

_____

Are mirror movements present? No Yes

_____

_____

Are any movements facilitated by…

Finger reflexes

Tactile stimulation

Righting reflexes

Proximal traction response

Co-ordination:

Gait: yes no

Bedridden

Can the patient sit

Can the patient stand

If walking…

With support of one

Slowly with an aid

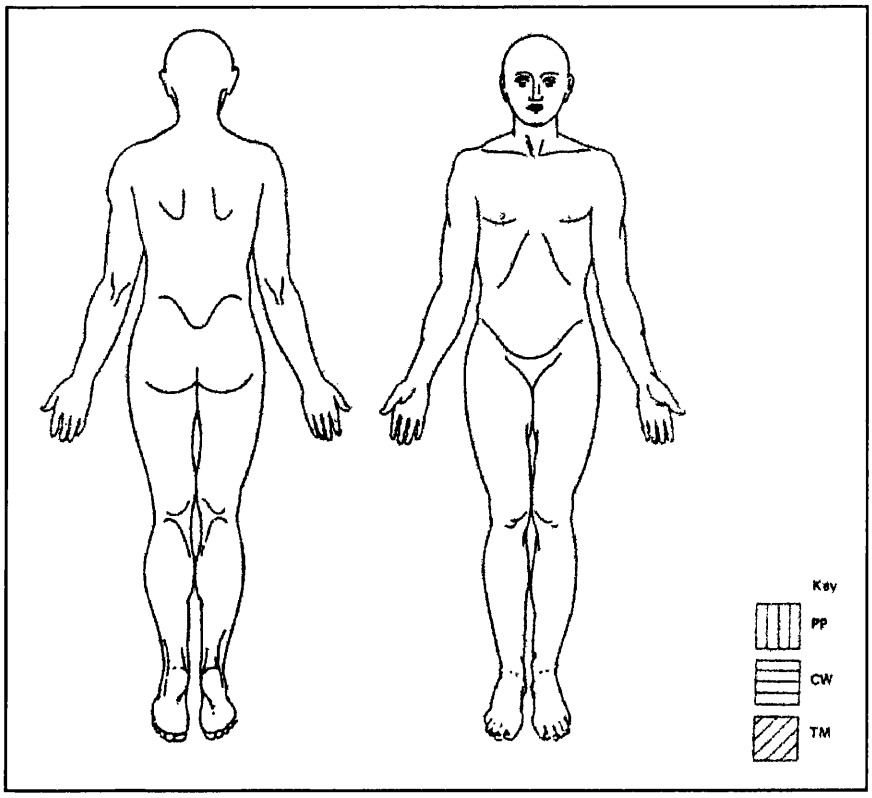
Normal

10m timed walk

Balance (OCS)

0.0 = walks 10 feet, no help
0.4 = stands unsupported for 1 min
0.8 = maintains sitting position
1.2 = no sitting balance

Sensory Examination



Visuopspatial Assessment	yes	no
Sensory inattention		
Visual inattention		
Agnosia		
Apraxia		

Describe:

Draw a clockface

Summary

Diagnosis

Investigations:

FBC

ESR

COAG: PT: INR: APTT: TT:

U & E: Na K Urea Creat

LFTS: Alb Bili APos GGT AST ALT

GLU (fasting)**:** **GLU** (random)**:**

TFTS: T4: T3: TSH:

LIPID PROFILE: CHOL: TGS: HDL CHOL:

LDL CHOL:

ECG:

CXR:

ECHO:

CT BRAIN: (CONTRAST)

MRI BRAIN:

Arterial territory ……

Cortex involved	no	yes	
Internal capsule involved	no	yes	[posterior anterior genu]
Corticospinal tract involved	no	yes	site……
Leukoaraiosis	no	yes	

Other structures ……
……
……
……

DW MRI

MRA EXTRACRANIAL / INTRACRANIAL:

COLOUR DOPPLER U / S:

FOLLOW UP ASSESSMENT: STROKE RECOVERY STUDIES

Patient Name:

Date (Time):

Days since stroke:

Assessment performed by:

A: BEDSIDE TESTING

10 Metre walk

1. Comments:
2.
3.

Average Speed (m/s)

Tone: Modified Ashworth Scale

0 No increase in muscle tone
1 Slight increase in muscle tone, manifested by catch and release, or by minimal resistance at the end of motion when the affected part is moved in flexion or extension
2 Slight increase in muscle tone, manifested by catch, followed by minimal resistance throughout the remainder (less than half) of the remainder of movement
3 More marked increase in muscle tone through most of ROM, but affected parts move easily.
4 Considerable increase in muscle tone, passive movement difficult
5 Affected part rigid in flexion or extension.

RUL _____ LUL _____

RLL _____ LLL _____

Comments _____

MOTRICITY INDEX

ARM	RIGHT	LEFT
1. Pinch grip	_____	_____
2. Elbow flexion	_____	_____
3. Shoulder abduction	_____	_____
TOTAL (+1)	_____	_____
LEG		
1. Ankle dosiflexion	_____	_____
2. Knee extension	_____	_____
3. Hip Flexion	_____	_____
TOTAL (+1)	_____	_____
SIDE SCORE	☐	☐

Scoring

Test 1 (pinch grip)

0	No movement
11	Beginnings of prehension (any movement)
19	Grips cube, unable to hold against gravity
22	Grips cube against gravity, but not against weak pull
26	Grips cube against pull, but weaker than other side
33	Normal pinch grip

Test 2-6

0	No movement
9	Palpable contraction, no movement
14	Movement seen, but not full range / not vs gravity
19	Full range against gravity but not resistance
25	Movement against resistance, but weaker than other side
33	Normal

Guidelines

Pinch Grip
Patient grips 2.5cm cube between thumb and forefinger. Cube should be on flat surface

Elbow Flexion
Elbow flexed to 90°, forearm horizontal, upper arm vertical. Patient asked to bend elbow so that hand touches shoulder. Examiner resists with hand or wrist. Monbitor biceps

14 – if no movement seen hold elbow so that arm is horizontal

Shoulder Abduction
With elbow fully flexed and against chest, patient asked to abduct arm. Monitor contaction of the deltoid. Movement of the shoulder girdle does not count, there must be movement of the humerus in relation to the scapula.

19 – abduction past the horizontal

Ankle Dorsiflexion
Feet relaxed and in plantar flexed position. Patient asked to dorsiflex foot. Monitor tibialis anterior.

14 – not full range of dorsiflexion

Knee Extension
Feet unsupported, knee at 90°. Patient asked to straighten knee to touch examiners hand held level with the knee. Monitor contractions of the quadriceps

14 – less than 90° of full extension
19 – knee fully extends but easily pushed down

Hip Flexion
Sitting with hip bent at 90°. Patient asked to lift knee to chin. Patient should not lean back placing hands behind back. Monitor contraction of ilio-psoas.

14 – less than full range as compared to passive movement
19 – fully flexed but easily pushed down.

(Tests performed whilst sitting if possible)

ORPINGTON PROGNOSTIC SCALE

A. Motor Deficit

Test motor deficit whilst patient lying down and attempting to flex shoulder to 90°

0.0 Normal power (grade 5)
0.4 Reduced power (grade 4)
0.8 Movement against gravity (grade 3)
1.2 Movement with gravity eliminated (grade 1-2)
1.6 No movement (grade 0)

B. Proprioception

Locate thumb with eyes closed

0.0 Accurate
0.4 Slight difficulty
0.8 Finds thumb via arm
1.2 Unable to find arm

C. Balance

0.0 Walks 10 feet without help
0.4 Maintains standing position unsupported for 1 min
0.8 Maintains sitting position
1.2 No sitting balance

D. Cognition

One point for each correct answer

Age of patient _____
Time to nearest hour _____
(now state an address with no. and street name)
Name of hospital _____
Year _____
DOB _____
Month _____
Years of WWII _____
Name of Prime Minister _____
Count backwards (20-1) _____
What is the address I told you _____

0.0 score = 10
0.4 score = 8-9
0.8 score = 5-6
1.2 score = 0-4

TOTAL = 1.6 + A + B + C + D =

BARTHEL ADL INDEX

Continence of bowels:
0 = incontinent (or needs enemata)
1 = occasional accident (once a week)
2 = continent

Continence bladder:
0 = incontinent, or catheterised and unable to manage alone
1 = occasional accident (maximum: once daily)
2 = continent

***Grooming*:**
0 = Needs Help With Personal Care
1 = Independent Face/ Hair/ Teeth/ Shaving (Implements Provided)

***Getting On and Off Toilet*:**
0 = Dependent;
1 = Needs Some Help, But Can Do Something Alone
2 = Independent (On And Off, Dressing, Wiping)

Feeding:
0 = Unable
1 = Needs Help Cutting, Spreading Butter Etc
2 = Independent

Transfer (bed to chair and back):
0 = Unable, No Sitting Balance
1 = Major Help (One Or Two People, Physical), Can Sit
2 = Minor Help (Verbal Or Physical)
3 = Independent

Walking On Level Surface:
0 = Immobile
1 = Wheelchair Independent, Including Corners
2 = Walks With Aid Of One Person (Verbal Or Physical)
3 = Independent (But May Use Any Aid e.g. Stick)

Dressing and undressing:
0 = dependent
1 = needs help but can do about half unaided
2 = independent (including buttons, zips, laces etc.)

Ascending and descending stairs:
0 = unable;
1 = needs help (verbal / physical, carrying aid)
2 = independent

Bathing Self:
0 = Dependent
1 = Independent (Or In Shower)

Bowels (preceding week)
- If needs enema from nurse, then 'incontinent'
- Occasional = once a week

Bladder (preceding week)
A catheterized patient who can completely manage the catheter alone is regarded as 'continent'.

Grooming (preceding 24–48 hours)
Refers to personal hygiene: doing teeth, fiting false teeth, doing hair, shaving, washing face. Implements can be provided by helper.

Toilet use
- Should be able to reach toilet/commode, undress sufficiently, clean self, dress and leave
- With help = can wipe self and do some of above

Feeding
- Able to eat any normal food (not only soft food). Food cooked and served by others but not cut up
- Help = food cut up, patient feeds self

Transfer
- From bed to chair and back
- Dependent = *no* sitting balance (unable to sit); two people to lift
- Major help = one strong/skilled, or two normal people—can sit up
- Minor help = one person easily, *or* needs any supervision

Mobility
- Refers to mobility about house or ward, indoors. May use aid. If in wheelchair, must negotiate corners/doors unaided
- Help = by one untrained person, including supervision and moral support

Dressing
- Should be able to select and put on all clothes, which may be adapted
- Half = help with buttons, zips, etc. (check!), but can put on some garments alone

Stairs
- Must carry any walking aid used to be independent

Bathing
- Usually the most difficult activity
- Must get in and out unsupervised and wash self
- Independent in shower = 'independent' if unsupervised and unaided

RANKIN

0	No symptoms at all
1	No significant disability, despite symptoms; able to carry out all usual duties and activities
2	Slight disability; unable to carry out all previous activities but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance.
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention

B: FUNCTIONAL ASSESSMENT - UPPER LIMB

NINE HOLE PEG TEST

Patients asked to place nine pegs in holes. Record time to place nine pegs or number of pegs placed in 50 seconds

LEFT HAND (circle affected side)				RIGHT HAND			
Test	**No. Pegs**	**Time**	**Pegs/sec**	**Test**	**No. Pegs**	**Time**	**Pegs/sec**
1.	_____	____	_____	1.	_____	____	_____
2.	_____	____	_____	2.	_____	____	_____
3.	_____	____	_____	3.	_____	____	_____
Mean Score							

(Mean affected / Mean unaffected) x 100% =

GRIP STRENGTH

LEFT HAND (circle affected side)		RIGHT HAND	
Test	**Force**	**Test**	**Force**
1.	_____	1.	_____
2.	_____	2.	_____
3.	_____	3.	_____
Max score	_____		_____

(Max affected / Max unaffected) x 100% =

Was patient able to release grip? Yes / No

ACTION RESEARCH ARM TEST

		Left	Right
Grasp			
1 ____	Block, wood, 10-cm cube (If score = 3, total = 18 and go to *Grip*) Pick up a 10-cm block		
2 ____	Block, wood, 2.5-cm cube (If score = 0, total = 0 and go to *Grip*) Pick up 2.5-cm block		
3 ____	Block, wood, 5-cm cube		
4 ____	Block, wood, 7.5-cm cube		
5 ____	Ball (Cricket), 7.5-cm diameter		
6 ____	Stone 10 × 2.5 × 1 cm		
Coefficient of reproducibility = 0.98 Coefficient of scalability = 0.94		☐	☐
Grip			
1 ____	Pour water from glass to glass. (If score = 3, total = 12 and go to *Pinch*)		
2 ____	Tube 2.25 cm (If score = 0, total = 0 and go to *Pinch*)		
3 ____	Tube 1 cm × 16 cm		
4 ____	Washer (3.5 cm diameter) over bolt		
Coefficient of reproducibility = 0.99 Coefficient of scalability = 0.94		☐	☐
Pinch			
1 ____	Ball bearing, 6 mm, 3rd finger and thumb (If score = 3, total = 18 and go to *Grossmt*)		
2 ____	Marble, 1.5 cm, index finger and thumb (If score = 0, total = 0 and go to *Grossmt*)		
3 ____	Ball bearing 2nd finger and thumb		
4 ____	Ball bearing 1st finger and thumb		
5 ____	Marble 2nd finger and thumb		
6 ____	Marble 1st finger and thumb		
Coefficient of reproducibility = 0.99 Coefficient of scalability = 0.98		☐	☐
Grossmt (Gross movement)			
1 ____	Place hand behind head (If score = 3, total = 9 and finish)		
2 ____	(If score = 0, total = 0 and finish)		
3 ____	Place hand on top of head		
4 ____	Hand to mouth		
Coefficient of reproducibility = 0.98 Coefficient of scalability = 0.97		☐	☐
Total		☐	☐

Scoring

3 = performs test normally
2 = completes test but takes abnormally long time or has great difficulty
1 = performs test partially
0 = can perform no part of the test

C: COGNITIVE FOLLOW UP

AUDITORY VERBAL COMPREHENSION

Answers should be yes or no, establishing first whether the response will be verbal or gestural (including eyeblink). Avoid nodding or commenting on specific items. Self corrections by the patient are counted. If response is ambiguous then repeat the instructions and question. If response still ambiguous score 0. Score 3 for each correct answer.

1. Is your name Smith? (no should be correct)
2. Is your name Brown? (no should be correct)
3. Is your name _____?
4. Do you live in Toronto? (no should be correct)
5. Do you live in _____?
6. Do you live in Windsor? (no should be correct)
7. Are you a man/woman? (yes should be correct)
8. Are you a doctor? (no should be correct)
9. Am I a man? (yes should be correct)
10. Are the lights on in this room? (lights are on)
11. Is the door closed? (door is closed)
12. Is this a hotel?
13. Is this a hospital? (yes should be correct)
14. Are you wearing red pyjamas? (no should be correct)
15. Will paper burn in fire?
16. Does March come before June?
17. Do you eat a banana before you eat it?
18. Does it snow in July?
19. Is a horse larger than a dog?
20. Do you cut the grass with an axe?

Patient score

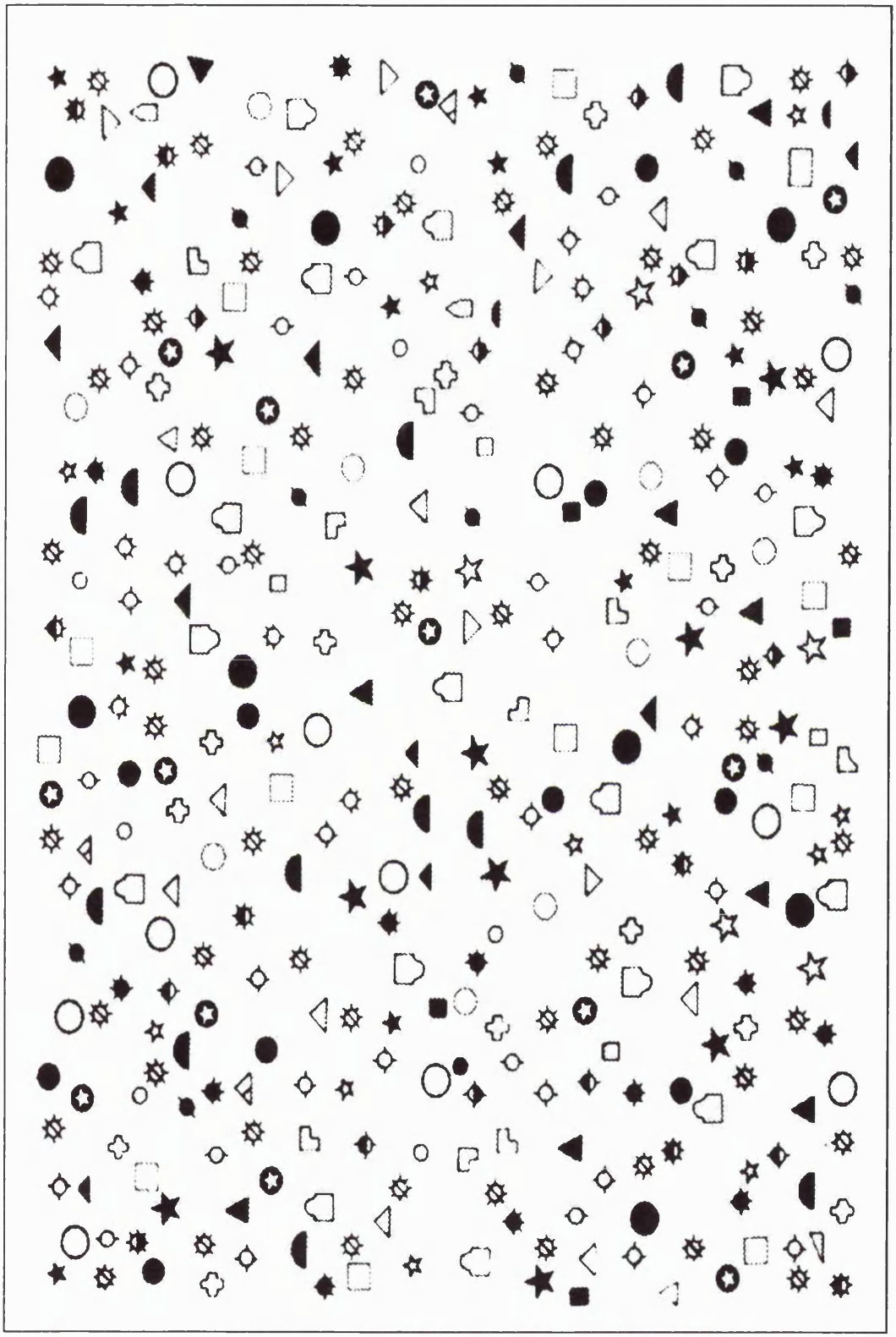
Number Cancellation

1	6	1	6	5	7	0	4	8	1	7	1
4	6	5	3	2	9	7	3	4	6	4	2
7	7	6	3	6	2	5	8	6	0	5	9
6	3	8	9	5	2	7	7	2	3	6	1
6	3	9	0	8	0	3	8	4	2	7	1
7	0	0	4	8	1	0	5	5	0	0	1
0	9	3	5	0	2	5	4	5	1	9	6
7	5	5	8	2	9	2	4	2	3	2	5
8	9	9	7	9	1	2	9	2	9	0	7
3	4	4	9	2	2	5	2	9	6	8	9
1	7	1	1	0	6	9	1	2	4	3	8
0	6	8	3	5	9	7	2	6	1	8	0
7	0	9	9	2	4	6	4	1	1	3	8
6	5	2	7	2	3	4	0	3	7	8	4
4	8	5	3	7	1	1	1	4	1	8	2
3	7	0	0	4	5	9	8	5	4	5	2
2	6	3	4	4	0	1	3	6	0	3	8
8	6	6	1	0	5	6	6	1	8	7	6
1	1	1	8	6	1	9	0	9	0	6	3
5	7	3	2	0	6	3	9	9	5	7	5
8	1	8	9	4	2	3	4	0	0	4	9

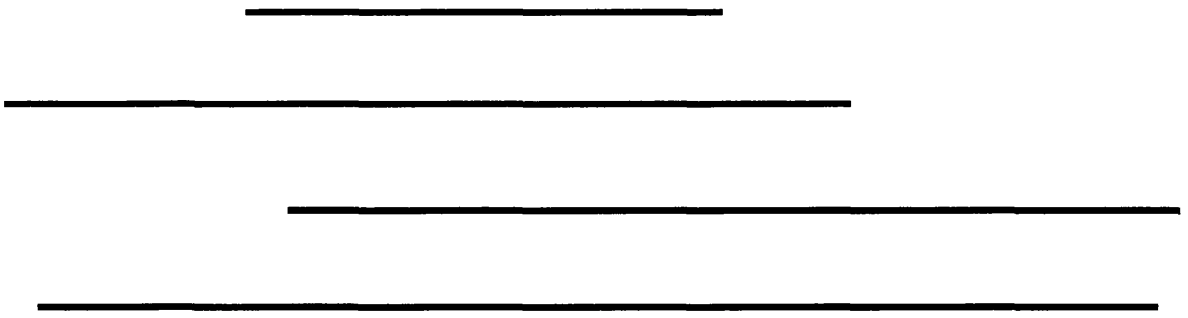
Letter Cancellation

D	A	E	E	B	A	C	C
B	E	D	E	A	B	E	A
B	B	D	B	C	B	E	A
B	B	A	C	D	D	B	B
B	C	D	A	A	A	B	D
E	E	C	E	B	A	E	D
D	D	A	A	C	C	E	B
B	C	D	C	D	E	D	E
C	A	A	A	D	A	B	C
D	E	E	D	E	B	B	C
D	D	A	C	C	A	E	A

Shape Cancellation



Line Bisection



PRAXIS

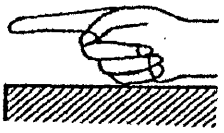
Phrase each command in these terms:
"Imagine that you are holding a comb in your hand and you are combing your hair. Shoe me what you would do"

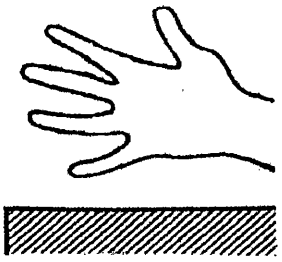
Scoring
3 Good performance
2 Approximate performance OR good performance on imitation OR body part as object
1 Approximate performance on imitation OR if performed with actual object

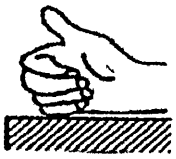
	COMMAND	IMITATION	OBJECT	
				Score
Put your tongue out				
Close your eyes				
Whistle				
Sniff a flower				
Blow out a match				

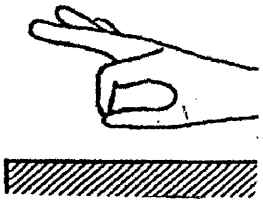
	COMMAND		IMITATION		OBJECT		Score	
	Right	Left	Right	Left	Right	Left	Right	Left
Make a fist								
Salute								
Wave Goodbye								
Scratch your head								
Snap your fingers								
Use a comb								
Use a toothbrush								
Use a spoon to eat								
Use a hammer								
Use a key								

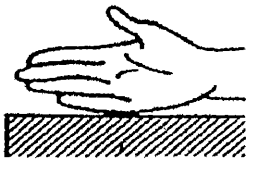
Copy the following hand gestures

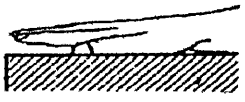












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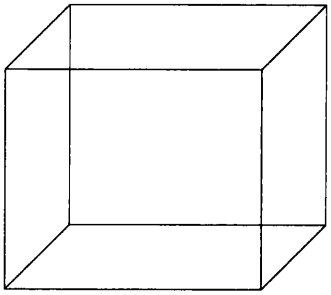	
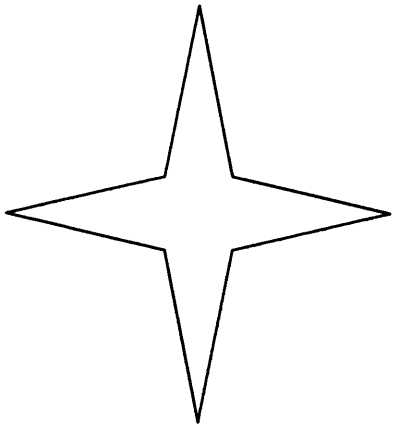	
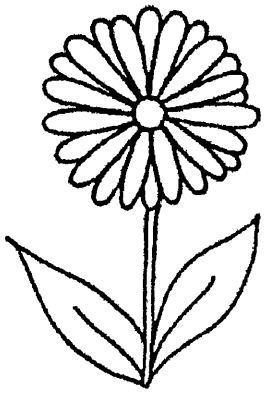	

Further Comments:

APPENDIX II

INDIVIDUAL PATIENT LONGITUDINAL OUCOME SCORES

This appendix contains all the outcome scores collected on each of the eight stroke patients participating in the longitudinal study (chapter 7).

PATIENT 1 OUTCOME SCORES	days post-stroke					
	11	16	22	29	91	200
Barthel	12	18	19	20	20	20
Rankin	3	2	2	1	1	1
Orpington Prognostic Scale	2.4	2	2	1.6	1.6	1.6
Action Research Arm Test	33	51	53	56	57	57
Grip Strength (% unaffected side)	41.2	46.1	81.1	78.9	85.2	88.6
Motricity Index - arm	67	77	77	92	100	100
Motricity Index - leg	73	84	84	92	100	100
9-hole peg test (pegs/sec) - affected	0.02	0.07	0.12	0.3	0.47	0.55
9-hole peg test (pegs/sec) - unaffected	0.53	0.54	0.64	0.68	0.72	0.74
Timed 10m walk (m/s	0.45	0.48	0.55	0.85	1.06	1.33

PATIENT 2 OUTCOME SCORES	days post-stroke						
	10	16	23	30	37	125	197
Barthel	19	19	20	20	20	20	20
Rankin	2	2	1	1	1	1	1
Orpington Prognostic Scale	2	2	1.6	1.6	1.6	1.6	1.6
Action Research Arm Test	56	56	57	57	57	57	57
Grip Strength (% unaffected side)	48.1	80.1	79.2	87.4	93.8	96.3	101.2
Motricity Index - arm	93	93	100	100	100	100	100
Motricity Index - leg	92	100	100	100	100	100	100
9-hole peg test (pegs/sec) - affected	0.23	0.28	0.34	0.4	0.42	0.47	0.46
9-hole peg test (pegs/sec) - unaffected	0.64	0.62	0.65	0.62	0.59	0.62	0.61
Timed 10m walk (m/s	O.4	1.01	0.98	1.03	1.07	1.05	1.4

PATIENT 3 OUTCOME SCORES	days post-stroke						
	9	12	18	24	113	160	207
Barthel	19	20	20	20	20	20	20
Rankin	2	1	1	1	1	1	1
Orpington Prognostic Scale	2	1.6	1.6	1.6	1.6	1.6	1.6
Action Research Arm Test	56	57	57	57	57	57	57
Grip Strength (% unaffected side)	71.3	79.1	77.8	83.1	84.5	91.2	93.1
Motricity Index - arm	93	100	100	100	100	100	100
Motricity Index - leg	92	100	100	100	100	100	100
9-hole peg test (pegs/sec) - affected	0.32	0.49	0.51	0.55	0.64	0.59	0.68
9-hole peg test (pegs/sec) - unaffected	0.51	0.65	0.61	0.57	0.6	0.55	0.63
Timed 10m walk (m/s	1.01	1.22	1.36	1.44	1.59	1.54	1.6

PATIENT 4 OUTCOME SCORES	days post-stroke									
	14	20	27	33	41	47	62	76	112	174
Barthel	12	13	14	14	14	15	16	19	20	20
Rankin	4	4	4	4	4	3	3	2	2	2
Orpington Prognostic Scale	3.2	2.4	2.4	2.4	2.4	2	2	2	2	2
Action Research Arm Test	53	57	57	57	57	57	57	57	57	57
Grip Strength (% unaffected side)	47.9	58.1	64.3	72.5	70	78.5	97.1	98.2	98.5	98.7
Motricity Index - arm	72	72	84	84	84	84	84	84	84	92
Motricity Index - leg	64	78	84	84	84	84	84	84	84	100
9-hole peg test (pegs/sec) - affected	0.11	0.17	0.2	0.23	0.26	0.28	0.34	0.36	0.37	0.39
9-hole peg test (pegs/sec) - unaffected	0.35	0.37	0.4	0.04	0.44	0.43	0.44	0.44	0.43	0.45
Timed 10m walk (m/s	0	0	0	0	0	0.58	0.77	0.98	1.1	1.54

PATIENT 5 OUTCOME SCORES	days post-stroke					
	14	17	25	31	93	193
Barthel	19	19	20	20	20	20
Rankin	2	1	1	1	1	1
Orpington Prognostic Scale	2	2	1.6	1.6	1.6	1.6
Action Research Arm Test	56	57	57	57	57	57
Grip Strength (% unaffected side)	91.8	100.7	98.7	97	97.5	103.3
Motricity Index - arm	93	100	100	100	100	100
Motricity Index - leg	92	100	100	100	100	100
9-hole peg test (pegs/sec) - affected	0.56	0.64	0.67	0.7	0.59	0.73
9-hole peg test (pegs/sec) - unaffected	0.62	0.69	0.68	0.68	0.66	0.71
Timed 10m walk (m/s)	1.95	1.96	2.05	2.12	2.1	2.25

PATIENT 6 OUTCOME SCORES	days post-stroke									
	10	12	22	26	31	38	45	51	94	191
Barthel	18	18	18	18	19	19	19	19	20	20
Rankin	4	4	3	3	3	3	3	2	2	1
Orpington Prognostic Scale	2.4	2.4	2.4	2.4	2	1.6	1.6	1.6	1.6	1.6
Action Research Arm Test	54	54	57	57	57	57	57	57	57	57
Grip Strength (% unaffected side)	71.3	72	98.8	94.4	96.1	99.2	96.2	98.5	99.1	105
Motricity Index - arm	77	77	77	85	92	92	100	100	100	100
Motricity Index - leg	73	73	84	84	84	84	84	84	92	100
9-hole peg test (pegs/sec) - affected	0.2	0.23	0.25	0.25	0.29	0.32	0.4	0.39	0.45	0.48
9-hole peg test (pegs/sec) - unaffected	0.67	0.63	0.59	0.56	0.52	0.63	0.57	0.56	0.6	0.61
Timed 10m walk (m/s	0	0	0.3	0.32	0.41	0.52	0.53	0.59	1.05	1.02

PATIENT 7 OUTCOME SCORES	days post-stroke									
	14	**24**	**31**	**35**	**46**	**53**	**66**	**160**	**250**	**370**
Barthel	11	14	15	15	17	19	20	20	20	20
Rankin	4	4	4	4	3	2	2	1	1	1
Orpington Prognostic Scale	3.6	3.2	2.8	2.8	2	2	2	1.6	1.6	1.6
Action Research Arm Test	13	24	27	29	52	55	56	57	57	57
Grip Strength (% unaffected side)	3.2	14.1	18.8	20.6	69.3	88.6	90.4	91.7	97.5	100.9
Motricity Index - arm	62	62	67	71	76	76	76	100	100	100
Motricity Index - leg	40	54	63	63	77	77	77	100	100	100
9-hole peg test (pegs/sec) - affected	0	0	0.02	0.04	0.11	0.23	0.33	0.49	0.5	0.57
9-hole peg test (pegs/sec) - unaffected	0.72	0.6	0.64	0.62	0.62	0.63	0.61	0.6	0.62	0.73
Timed 10m walk (m/s)	0	0	0	0	0	0	0.5	1.32	1.4	1.68

PATIENT 8 OUTCOME SCORES	days post-stroke							
	10	**17**	**25**	**33**	**70**	**173**	**240**	**312**
Barthel	14	14	18	20	20	20	20	20
Rankin	4	3	2	2	1	1	1	1
Orpington Prognostic Scale	2.8	2.8	2	2	1.6	1.6	1.6	1.6
Action Research Arm Test	28	35	46	47	57	54	57	57
Grip Strength (% unaffected side)	49.1	48.1	46.8	49.5	50.4	79.8	100.3	106.9
Motricity Index - arm	73	73	77	84	93	100	100	100
Motricity Index - leg	92	92	100	100	100	100	100	100
9-hole peg test (pegs/sec) - affected	0	0	0	0.04	0.05	0.44	0.47	0.47
9-hole peg test (pegs/sec) - unaffected	0.55	0.62	0.69	0.7	0.73	0.67	0.69	0.7
Timed 10m walk (m/s)	0	0	0.7	1.2	1.29	1.56	1.53	1.4

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