

Thesis submitted for the degree of
Doctor of Medicine
at the University of Leicester

By

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University of Leicester

November 2004

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Abstract

Functional Magnetic Resonance Imaging Studies In Focal Dystonia

Stephen Butterworth

The work described in this thesis was undertaken between 1998 and 2001 at the Magnetic Resonance Centre, School of Physics and Astronomy, University of Nottingham.

The thesis explores the cortical pathophysiology of focal dystonia by utilising the relatively new and powerful tool of Functional Magnetic Resonance Imaging (fMRI).

The thesis is presented in seven chapters. Chapter one outlines the clinical condition of focal dystonia and its place amongst the larger group of dystonias. It includes a review of the current literature on dystonia pathophysiology. Chapter two describes NMR, functional imaging and the methods used in the thesis. Chapter three presents a study into the cortical activations associated with a non-dystonia inducing task. Chapter four presents the changes in cortical activations associated with increasing task difficulty, including dystonia-inducing tasks. Chapter five presents a study into cortical sensory activations in focal dystonia, following peripheral digital stimulation. Chapter six describes pilot investigations into the spatial distribution of discrete motor movements. Chapters three to six include a full discussion of the results in context with the existing literature.

Chapter seven summarises the results of the studies and discusses theories of dystonia pathophysiology. This chapter also deals with the limitations of the methodology and the possible future directions of study.

Acknowledgements

I am extremely grateful to the local branch of the Dystonia Society and all the patients who gave up their time to participate in the studies.

Many thanks also to the Medical Research Council whose grant funded this research project.

Thanks also to all at the 'Magres' centre for their help and inspiration and for trusting me with the scanner.

In addition many thanks to my long suffering supervisors Guy Sawle, Peter Morris and Graham Cherryman.

A Special thanks to Dr Sue Francis for her invaluable help without which I'm sure this thesis would not have been completed.

Finally, a multitude of thanks to Liz for her never-failing encouragement.

Chapter One

Dystonia & The Focal Dystonias

1.1. Introduction to Dystonia

Dystonia is a descriptive word for a range of conditions characterised by sustained muscle contraction that causes twisting movements or abnormal postures. It is variable in its expression and severity (see classification below) ranging from a completely disabling condition to only being manifest during specific actions, causing minimal disability.

With the advent of botulinum toxin as symptomatic therapy for dystonia, a growing number of patients are now seeking help for their dystonia. Over the past few years there has also been a revival in surgical treatment of dystonia especially with the development of deep brain stimulation. Most patients with dystonias have a normal life expectancy, so as the population ages there is an ever-growing number of sufferers. This new found ability to relieve some of the symptomatology in dystonic patients has given rise to a growing interest in the natural history and pathophysiology of dystonia. It is hoped that this heightened interest will lead to a greater understanding of the condition and to potential new treatments.

1.2. Classification of Dystonias

Focal dystonias are a subgroup of primary dystonias in a classification that is complex and ever expanding. Perhaps the best way of classifying dystonia was introduced by Fahn and Marsden in 1998 (Fahn 1998). In this classification the dystonias are divided into four groups:

- 1) Primary dystonia. This occurs in patients with no evidence of structural abnormality in the CNS. Tremor may or may not be present. The distribution of the disease maybe generalised or focal. Generalised dystonia is a group synonymous with idiopathic torsion dystonia or dystonia musculorum deformans (DMD). Focal dystonias include writer's cramp and other occupational cramps, blepharospasm and spasmodic torticollis. In this latter group the dystonia is limited to a particular site of the body or group of muscles.
- 2) Dystonia-plus syndromes. These are defined as dystonia in combined with other clinical features such as myoclonus (myoclonic dystonia) and parkinsonism (Dopa-responsive dystonia).
- 3) Secondary dystonia. These are dystonias with a demonstrable causative structural lesion or an exogenous metabolic cause such as drugs.
- 4) Heredodegenerative dystonia. This occurs when dystonia is part of underlying brain degeneration such as dystonia seen as part of Parkinson's disease and Huntington's chorea.

1.3. Terms and definitions

The commonest cause of dystonia is secondary to drugs; following this the most numerous are the primary dystonias. Although other types of dystonia may provide a fascinating insight into the nature and aetiology of the condition, the discussion will

mainly be centred on the primary dystonias. It is a fact that a lot of the research into dystonia has been performed on primary dystonia, often with a mixture of patients with focal and generalised primary dystonias. As more information about the aetiology of these conditions becomes available it is clear that although the conditions are similar phenotypically they may be pathologically different.

As the following thesis will concentrate on studying 'arm' dystonias it is worthwhile defining the differing types. Writer's cramp is an example of focal dystonia; the dystonia manifests itself as an abnormal and inappropriate muscular contraction only when the subject is writing. The pattern of the muscles involved is variable and may involve single opposing muscles or, more commonly, a number of muscles. In some cases, writing is the only action that will cause the hand to cramp (simple writer's cramp); in other cases a variety of actions may precipitate the dystonia contractions such as sewing, typing etc (dystonic writer's cramp). Focal arm dystonia is a term used to describe dystonic cramps limited to one arm but not just the muscles involved in hand movements.

1.4. Epidemiology

1.4.1. Incidence and Prevalence

A number of attempts have been made to quantify the incidence and prevalence of primary dystonia. Only one estimate of the incidence of dystonia has been presented, which recorded a rate of 2 per million for early onset (mainly generalised) primary dystonia, and 24 per million for late onset (mainly focal and segmental) (Nutt 1988). There have, however, been numerous prevalence studies (Eldridge 1970, Zilber 1984, Li 1985, Nutt 1988, Gimenez-Roldan 1988, Kandil 1994, Risch 1995, Nakashima 1995, Claypool 1995, Duffey 1998, ESDE collaborative group 2000,

Muller 2002, Matsumoto 2003, Le 2003, Pekmezovic 2003, Butler 2004). These studies have differed in their design, from service-based studies, to community studies. These studies have reported rates for generalised dystonia between 2 and 50 per million for generalised dystonia, and between 30 and 7320 per million for focal/segmental. Defasio *et al*, in a review of the methodological basis of these studies, have suggested that the higher estimates appear to come from the methodologically more robust studies (DeFazio 2004)

1.4.2. Sex Differences

In a review of an established database of patients and of the literature, Soland *et al* looked at differences in sex prevalence of the focal dystonias (Soland 1996). On the whole, women are more affected than men in cranial (1.92:1) and cervical (1.6:1) dystonias. On the other hand, focal dystonia have a higher prevalence in men (1.5M:1F). Occupational dystonias appear to have sex prevalence relating to the occupations involved (more women with typist cramp but more men with golfer cramp).

1.4.3. Ethnic differences

Genetically determined dystonias, as expected have higher rates in isolated populations such as the Ashkenazi Jews. Otherwise, dystonia appears to be described from all over the world.

1.5. Historical Aspects

There are no obvious descriptions of dystonia in antiquity; this fact leads us to the conclusion that the disorder didn't exist in the older world and is perhaps a disorder of the modern world. One of the first recognisable descriptions of dystonia appeared in the neurological literature in the early 1800's. It is of historical note that focal dystonias were described prior to generalised dystonias. J.H. Kopp produced a

detailed description of writer's cramp in his 1836 monograph (Kopp 1836). In this, he detailed a description of a fifty-year-old man who:

"suffered over one year with difficulties in writing, an activity that had previously been one of his talents...he has normal strength and he is able to hold up a heavy chair in the air with the hand. If, however, he takes a feather into his hand in order to write he develops an uncomfortable, trembling and painful sensation in the thumb and second finger. This discomfort increases during writing and forces him often to lay down the feather... "

This description highlights a number of cardinal features specific to focal dystonia. Often it develops in people who perform a particular task regularly and with some skill. The problems are progressive and the cramp will eventually make performing the task intolerable, which leads to the patients being alienated from the task. The description also highlights the otherwise normal functioning of the hand in terms of strength and ability to perform other tasks.

Generalised dystonia was originally described under the title of athetosis, a term used primarily by W.A.Hammond. He proposed that the characteristics of the athetosis, namely the sustained twisting nature and the exacerbation by action, perhaps were caused by abnormalities in the basal ganglia. Hammond's son in 1890 presented a paper on the autopsy findings of one of his father's cases:

"the motor tract was not implicated in the lesion and claimed that this case was further evidence of his theory that athetosis was caused by irritation of the thalamus, the striatum or the cortex, and not by a lesion of the motor tract" (Hammond 1890).

This lack of an obvious pathological substrate in 'athetosis' proved frustrating for many researchers in the 1900's. In 1908 Schwalbe wrote a thesis on 'Tonic cramps

with Hysterical Symptoms' in which he described generalised dystonia, eluding to its pattern of progression and its heritability (Schwalbe 1908). He also, as the name of the thesis implies, introduced an alternative aetiology for the condition, namely a psychiatric one. There are a number of characteristics of dystonia, such as the bizarre contortions, the occasional spontaneous remission, its exacerbation by movement and indeed the ability for focal dystonias to be task specific, that make a solely organic aetiology for the condition difficult to believe. These characteristics combined with *geste antagoniste*, an odd phenomenon that sees the dystonic contractions overcome or prevented by often the lightest touch, lead to confusion about the aetiology that underpins focal dystonias. How patients could perform the most complicated hand tasks but be unable to write was curious, as was the ability of some patients to write on a blackboard but not on paper at a desk. These puzzling paradoxes meant that for long periods of the 1900's, both generalised and focal dystonias were seen and treated by psychiatrists who offered Freudian hypotheses to explain the symptomatology. Oppenhiemer introduced the term 'dystonia' in 1911. He suggested that there was essentially a generalised abnormality of tone with coexistent hypotonia and hypertonia (Oppenhiemer 1911).

In 1944, Herz described a number of cases of generalised dystonia with associated multiple picture frames of the condition and electromyographical findings (Herz 1944). Despite this, a clinico-pathological correlation remained obscure and research again waned until the 1980's. In 1982, Sheehy and Marsden confirmed the view that writer's cramp was a form of focal dystonia and not a psychiatric disorder (Sheehy 1982). Further to this, Marsden *et al* and Pettigrew *et al*, in 1985 took a different approach to the subject by looking at cases of hemidystonia and demonstrating that they were due to lesions in the contra-lateral putamen, caudate nucleus and posterior

thalamus (Marsden 1985, Pettigrew 1985). From this point on, there was resurgence in interest in the investigation of dystonia that continues at pace today.

1.6. Pathophysiology of Dystonia: Review of Literature

Investigations into the pathophysiology of dystonia are legion. Over the years, these investigations have involved a variety of techniques reflecting the developments in the tools available for neurological research. A review of these studies is undertaken below.

1.6.1. Neuropathological studies

Autopsy studies in primary dystonia are rare and pathological findings inconsistent. Most studies tend to show no neuropathology or any evidence of neuronal loss (Jellinger 1998, Hornykiewicz 1986, Zeman 1968). Some descriptions in genetically determined dystonia have shown some neuronal loss in the substantia nigra (pars compacta) (Zweig 1988) and gliosis in the putamen (Waters 1994). In focal dystonia either no abnormality or patchy neuronal loss and gliosis in the putamen and caudate (Altrocchi 1983) or changes in the brain stem and diencephalon have been described (Mark 1994).

More recently, neuropathological studies in four patients with clinically and genetically confirmed DYT1 dystonia, have been shown to display neuronal inclusions with positive staining for Torsin A. These inclusions were found in the cholinergic and other neurons in the pedunculopontine nucleus, cuneiform nucleus and griseum centrale mesencephali. In addition tau/ubiquitin immunoreactive aggregates were demonstrated in the substantia nigra pars compacta and locus coeruleus (McNaught 2004).

1.6.2. Neurochemical studies

There have only been a few neurochemical studies in dystonia. In a study of the major neurotransmitter systems of the brain in cases of generalised dystonia no abnormalities in the cortex or striatum were found (Hornykiewicz 1986). They did, however, describe some minor changes in noradrenaline and serotonin levels in the thalamus, subthalamus and dorsal raphe nucleus. The significance of these changes is not clear. In genetically determined dystonia a decrease in striatal dopamine has been described (Furukawa 2000, Augood 2002). In this latter study, there was also evidence for increased striatal dopamine turnover.

1.6.3. Clues from Secondary Dystonia.

Further insights into the potential pathology that results in dystonia have come from secondary dystonia. Various forms of dystonia have been described following episodes of demyelination (Burguera 1991), stroke (Maimone 1991), trauma (both central (Krauss 2002) and peripheral (Bhatia 1993 Jankovic 2001)), as part of a paraneoplastic syndrome (Golbe 1989, Pulst 1982), or a consequence of infectious illnesses (Gollomp 1987, Kim 1995, Dale 2001, Green 2002, Cardoso 2002) and in abnormal metabolic states (Pal 1999).

Investigations into patients with secondary dystonia have shown that the causative lesions are often found in the basal ganglia (Marsden 1985, Pettigrew 1985). Bhatia *et al* studied the functional and behavioural outcomes in 240 patients with lesions to different areas of the basal ganglia. They showed that dystonia was present in 36% of the patients with basal ganglia lesions (Bhatia 1994). Within this group, the most frequent sites affected were the lentiform nuclei (globus pallidus and caudate nuclei). In a further study, dystonia was also observed in 30% of patients with lesions in the

thalamus and subthalamic region (Lee 1994). Within the thalamus, it is thought that the posterior and midline nuclei are most commonly affected. Obviously, this is not the whole story as patients can readily have asymptomatic lentiform and thalamic lesions. A prospective review of 175 patients with thalamic haemorrhages (Chung 1996) didn't document any patients suffering with dystonia as a consequence of their bleed.

1.6.4. Animal studies

It has long been thought that occupational hand cramps are an example of focal dystonia (Sheehy 1982, Cohen 1988, Jankovic 1989, Marsden 1990). It is believed that the genesis of these cramps is associated with the frequency of a repetitive skilled movement in a susceptible individual. Owl monkeys (Byl 1996) have been trained to perform a number of skilled movements and then 'persuaded' to overuse these movements (performing 300 precise hand grips a day for a number of weeks). These animals subsequently developed abnormalities that have a number of similarities to focal dystonia (it was noted that the speed and the accuracy of these handgrips were reduced).

As part of these experiments, microelectrode exploration of the parts of the somatosensory cortex involved with hand representation showed abnormalities. The representations showed both expansion and blurring of the previously rigorous boundaries seen in the controls.

It was postulated that this cortical reorganisation was mediated via the regular and repetitive sensory stimulation that the precision handgrip task produced. The authors speculated that there may be a similar reorganisation of the motor cortex and that this produces 'motor focusing' problems, causing inappropriate muscle groups to be

activated and dystonia to ensue. It was suggested that, for some reason, this plastic reorganisation becomes fixed in a susceptible individual.

A second animal model for focal dystonia has been described, namely a rat model of blepharospasm (Schicatano 1997). This model was not based upon a programme of over-use but on a combination of an intrinsic biochemical abnormality (unilateral striatal injection of 6-OHDA which depletes dopamine and hence increases the excitability of the blink reflex) and extrinsic damage to peripheral nerves (unilateral lesion to the zygomatic branch of the facial nerve). Neither of these lesions alone caused spasms of lid closure, but in combination they did. It was postulated that this 'two factor' model of a traumatic insult on top of an existing subclinical biochemical abnormality could be used as a model of pathogenesis in dystonias.

Further studies with monkeys have involved the application of bicuculline (a GABA antagonist) to the cortex of monkeys (Matsumura 1991). GABA is known to be a neurotransmitter of inhibitory neurons. The effect of this drug was to produce co-contraction of agonist and antagonist muscles during hand movement, characteristic of dystonia.

1.6.5. Genetic studies

There are 15 different types of genetically determined dystonia currently described (De Carvalho 2002, Grotzsch 2002, Grimes 2002) Primary dystonia accounts for six of these fifteen. These have been labelled DYT 1,2,4,6,7 and 13. Of importance are DYT1, 6,7 and 13 and these will be discussed below.

1.6.5.1. DYT-1

Early onset primary torsion dystonia (EOPTD) is a generalised dystonia defined as occurring below the age of 28 years, has been studied extensively in both Ashkenazi

Jews (a population that is known to have an increased incidence of early onset primary torsion dystonia) and in other non-Ashkenazi kindreds. Studies on dystonia have suggested that it is inherited in an autosomal dominant fashion (Korczyn 1980, Bressman 1989, Pauls 1990, Feltcher 1990). Ozelius *et al* were the first to identify that a locus on chromosome 9 that was linked to EOPTD. This locus for the gene has been further refined to 9q32-34. This gene named DYT1 was originally found in non-Jewish American kindred (Ozelius 1989), but the same region has subsequently been linked to kindred's in Ashkenazi (Kramer 1990) and European populations (Warner 1993). It has been postulated that the EOPTD in the Ashkenazi population is due to a founder mutation effect (Risch 1995). It has been calculated that the mutation was introduced 350 years ago probably in Lithuania or Byelorussia. Screening of large cohorts of cases with EOPTD has shown that 75% of cases are heterozygous for the GAG deleted allele (Klein 2002).

The DYT1 gene has been further studied and was narrowed to a 150kb region (Ozelius 1997). The mutation responsible is a three base pair GAG deletion in the gene. This GAG deletion results in the loss of one of a pair of glutamic acid residues near the carboxyl terminus of the deduced 332 amino acid novel protein Torsin A. The function of Torsin remains somewhat of a mystery. It shares homology with the AAA⁺ superfamily of ATP proteins. These protein have been associated with a number of functions including protein folding and degradation, cytoskeletal dynamics, membrane trafficking, vesicle function and response to stress (Breakefield 2000). Torsin A localises to the endoplasmic reticulum (Kustedjo 2000) and is also found in vesicles throughout neural cells (Hewett 2000). Neuropathological studies have shown the presence of Torsin A positive staining inclusions cholinergic and other neurons at various sites in the brain (see section 1.6.1.) (McNaught 2004). The

authors speculated that this pathology supported the notion of impaired protein handling in DYT1 dystonia. It was also postulated that the distribution of these changes may explain some of the underlying pathophysiology of DYT1 dystonia.

1.6.5.2. DYT-6, DYT7 and DYT-13

Like EOPTD, later onset, focal, cervico-cranial dystonias appear to have an autosomal dominant inheritance pattern (Waddy 1991, Defaschio 1993, Leube 1997a) with a reduced penetrance of only 12%. It is still not clear however how much of the later onset and focal group of dystonias may have a non-genetical basis and therefore the figures for penetrance may well be higher than estimated.

DYT-6 was found in two families of Mennonite and Amish origin and the locus was identified on chromosome 8q21-22.16 (Almasy 1997). These patients has a relatively early onset (6-38yrs) but tend to have onset in the cervical or cranial muscles as opposed to the limb onset in the DYT1 phenotypic group. As the DYT6 group progresses there is severe disability of cervical and cranial musculature but generalisation of the dystonia is rare. The gene product has yet to be identified.

A further gene DYT7 (Leube 1996) has also been mapped in a family with cervical dystonia of German decent. This family had late onset focal dystonia. The locus for the gene was found to be chromosome 18p, its product is unknown. Further analysis of this gene in other cases both in Germany and Central Europe have shown it to be a common mutation in cases of cervical dystonia (Leube 1997b, Leube 1997c).

DYT-13 is the most recent dystonia locus to be described. It was described in an Italian family with prominent dystonia in the cranio-cervical and upper limb regions (Valente 2001). This locus maps to chromosome 1p36 and product for this gene is yet to be identified.

Despite the detection of these genes, it is clear that this is not the whole story, as a series of further families with mainly late onset dystonia of both cervical and limb onset have all tested negative for known DYT mutations (Brancati 2002).

1.6.6. Structural Brain Imaging

1.6.6.1. Magnetic Resonance Imaging (MRI)

MRI investigation in primary dystonia has failed to demonstrate any consistent abnormality (Rutledge 1988). In an attempt to demonstrate more subtle differences, twenty-two patients with torticollis were studied using a MRI at 2.0 Tesla. T2 values for various areas in the basal ganglia were calculated and it was found that in the patient group there were increases in T2 values in the lentiform nuclei bilaterally (Schneider 1994). This finding has been interpreted as reflecting cell loss and gliosis in these areas.

1.6.6.2. Magnetic Resonance Spectroscopy

MRS centred on the lentiform nucleus in 14 patients with focal hand dystonia and 12 controls, has been performed (Naumann 1998). No significant differences were found in the N-acetylaspartate(NAA)/creatine or lactate/creatine ratios suggesting no significant cell loss or metabolic disturbance in Dystonia. Contrary to this, others have described, when studying patients with torticollis, that the NAA/creatine and the NAA/Choline ratios to be significantly depressed in the striatum (Federico 2001). Little at this point can be inferred from these findings.

1.6.6.3. Transcranial sonography

Transcranial sonography is a relatively new technique that has been primarily used to assess the state of the cerebral arteries (Doppler mode). It can, however, produce a

two dimensional display of the brain parenchyma. Work by Becker and Berg has put this novel feature of transcranial ultrasound to some diagnostic use (Becker 2001). They have demonstrated abnormalities in dystonia where magnetic resonance imaging and computerised tomography have failed. Ultrasound evaluation of patients with primary adult onset sporadic dystonia has revealed a substantial increase in the echogenicity of the lentiform nucleus (LN) (Becker 1997, Naumann 1996). Patients studied with focal dystonia have shown increased LN echogenicity (predominantly in the contralateral nucleus). Patients have also been studied with blepharospasm and neuroleptic induced dystonia, and no increase in echogenicity has been found (Naumann 1996). The cause for the increased echogenicity remains obscure but may relate to excess levels of copper in the LN (Berg 1999, Becker 1999).

1.6.7. Neurophysiological studies

1.6.7.1. Electromyographical studies

A number of electrophysiological techniques have been employed in the study of study dystonia. Electrophysiological abnormalities have been described at rest in the most severe forms of dystonia. Some dystonias are only evident during action, and therefore a number of different paradigms have evolved involving contraction of the dystonic and other body parts.

Herz studied the involuntary dystonic postures in the 1940's and showed that the dystonic bursts were caused by several seconds of prolonged and continuous EMG activity (Herz 1944). It was additionally noted that there are a number of superimposed bursts of activity, these findings correlate to additional symptoms that can occur with dystonia such as tremor (Yanagisawa 1971, Jedynack 1991) or myoclonus (Obeso 1983). Further to this work in generalised dystonia, similar EMG

patterns have been demonstrated in a number of focal dystonias (Berardelli 1985), cervical dystonia (Podvinsky 1968), laryngeal dystonia (Van Pelt 1994) and writer's cramp (Cohen 1988).

Neurophysiological investigation of dystonia has also shown a number of abnormalities during the production of a willed or voluntary movement. During a simple rapid movement of a joint in a normal individual, a specific sequence of co-ordinated agonist and antagonist contractions must occur. For example, the extension of a finger requires the activation of the finger extensor muscles and the 'active' relaxation of the finger flexor muscles. This di- or tri-phasic activation (additional agonist burst to add precision) is seen with EMG in normal subjects (Berardelli 1996). In dystonia the EMG bursts responsible for the agonist and antagonist movements are prolonged and as a consequence there is greater degree of overlapping of the agonist and antagonist phases (Van der Kamp 1989). This is the electrophysiological demonstration of co-contraction.

In addition to the phenomenon of co-contraction there is also a lack of motor selectivity during movement; this is manifest as an overflow of motor activity during activation of the discrete muscles intended for the production of a specific movement. A good demonstration of this is seen in the work of van der Kamp *et al* which demonstrates excessive overflow of EMG detected activity into distant muscles (such as deltoid and pectoralis major) during forearm flexion (Van der Kamp 1989). Sequential arm movements have also been shown to be abnormal in Dystonia (Agostino 1992).

These two findings of 'co-contraction' and 'overflow' are thought to contribute to the slowness and increased variability of movements in patients with dystonia.

Co-contraction in dystonic subjects has been studied in more detail and it has been shown that the co-contraction in dystonia is neurophysiologically distinct from voluntary co-contraction (Farmer 1998). It was concluded that the dystonic co-contraction was produced by abnormal synchronisation of pre-synaptic inputs to the antagonist motor neurone pool. The level at which this abnormality occurs in the corticospinal tract (cortical or spinal) remains elusive.

1.6.7.2. Stimulation Studies

Using more complex electrophysiological techniques, dystonia has been studied further and abnormalities at the higher control levels have been deduced. Reciprocal inhibition is a normal phenomenon that helps control the co-ordination of agonist and antagonist muscles helping the sequencing of normal movements around a joint. Reciprocal inhibition of the H-reflexes in forearm flexors can be shown to occur in normal subjects by stimulation of the forearm extensor muscle afferents in the radial nerve. In the normal individual, the inhibition of antagonist muscles consists of a short (Day 1984) and longer lasting phases (Berardelli 1987) mediated by mainly peripheral spindle afferent fibres as well as possibly a centrally driven mechanism. Both of these inhibitory mechanisms have been studied in focal dystonia, and it has been shown there is a reduction in both phases (Chen 1995, Nakashima 1989). This lack of inhibition may explain the over activity of the antagonistic muscle and hence the genesis of co-contraction. It is postulated that the lack of reciprocal inhibition in dystonia is due to an abnormality in the control of spinal interneurons by descending tracts. Of further interest was a study examining this phenomenon in patients receiving botulinum toxin therapy, which showed restoration of long lasting reciprocal inhibition after injections with botulinum toxin (Priori 1995), arguing perhaps for peripheral mediation of the inhibition?

Unfortunately, the lack of reciprocal inhibition is not the whole explanation, as similar abnormalities can be seen in patients with writer's cramp in the non-affected arm (Chen 1995) and in the arms of patients with torticollis (Deuschl 1992) where the arm is not clinically affected. Why patients with similar abnormalities in reciprocal inhibition are not manifesting in the same way clinically is an interesting and as yet unanswered question. The authors of these papers reflect that the motor control mechanisms in focal dystonia were abnormal in a generalised pattern perhaps reflecting a basal ganglia outflow abnormality. It is perhaps also possible that these findings reflect a thresholding effect. It maybe that the abnormalities have to be of a certain magnitude or accompanied by another unidentified factor in order to be manifest.

1.6.7.2. Somatosensory Evoked Potentials (SSEP's)

Electrical stimulation of peripheral nerves, such as the median nerve, will produce a cortical correlation. This has been known for many years since original experiments on myoclonic epilepsy (Dawson 1947a&b).

SSEP's can be divided into a number of components dependent upon the time and site of their appearance in relation to the stimulation. The differing components of the SSEP are presumed to represent different areas of the brain that are becoming activated. The cortical origin of the N30 component of the SSEP is controversial and is thought to be the supplementary motor cortex (Mauguiere 1983) or the somatosensory cortex (Allison 1991, Hallett 1996). The N30 component has been studied widely in dystonia with varying results. It has been described as being increased in writer's cramp (Reilly 1992), normal (Nardone 1992) or small (Mazzini 1994) torticollis. It is clearly a confusing picture and probably reflects a number of

variables such as the type of patients studied and the technical difficulties of the technique (Hallett 1996).

Slow rate stimulation studies of the median nerve form different SSEP components. A study of the N140 and P190 components, thought to represent activity in the frontal regions including the SMA (Allison 1991) have both been shown to be deficient in dystonia (Grissom 1995). This finding is interpreted as reflecting underactivity of this area.

Further studies concentrating on the P37 (post central cortex) and N50 (precentral cortex) components of the SSEP (from tibial nerve stimulation) have shown significant enhancement of these components. This is thought to represent an abnormally excessive response of the motor cortex to the sensory stimulation (Tinazzi 1999a)¹⁰⁸.

SSEP's have also more recently been employed to study the somatotopic organisation of the somatosensory cortex in dystonia. Although this is not the supposed seat of the abnormalities in dystonia, there has been growing interest in the role of the sensory cortex in the production and maintenance of the dystonic abnormalities. In a study of individual digit stimulation and recording of the N20 component of the SSEP, a component which is thought to be generated by the anterior bank of the post-central gyrus) (Duff 1980). An abnormal organisation of individual digit representations (specifically thumb and little finger) in the sensory cortex has also been demonstrated (Bara-Jimenez 1998). The degree of this disorganisation also correlated with the severity of motor dysfunction in the dystonic patients. How the abnormal sensory organisation may cause an abnormal motor output was not speculated upon.

1.6.7.3. Movement evoked potentials

Electroencephalographic studies have been used for some time in the study of movement. Two slow rising pre-movement potentials were discovered in the 1960's. The contingent negative variation (CNV) was described as a negative potential recorded at the vertex during an interstimulus interval (Walter 1964). The Bereitschaftspotential (BP) (Kornhuber 1965) is a similar negative potential but is measured preceding self-paced movements.

1.6.7.3.1. Studies using the Contingent Negative Variation

The CNV is thought to reflect neural activity preparatory to a goal directed movement that takes into account sensory inputs (Regan 1989). This is in contrast to the BP that is independent of sensory input.

The late phase (1900ms after first stimulus) of the CNV has been studied and found to be abnormal in patients with torticollis (Kaji 1995a) and focal hand dystonia (Ikeda 1996). It is interesting that in the study by Kaji *et al*, the patients with torticollis (but no evidence of hand dystonia) were also asked to perform cued hand movements and no difference to controls could be found in the late CNV component prior to these movements. This was interpreted as evidence for task-specific amplitude loss, reflecting defective retrieval or retention of specific motor programmes in response to sensory stimuli.

1.6.7.3.1. Studies using the Bereitschaftspotential

The BP reflects the activity of the brain for the second prior to self paced movement. A number of different phases have been described (Deecke 1969) which are thought

reflect individual areas of the brain becoming activated. The terminology in this area has become confusing but is best divided as follows:

NS1 is the first phase of the Bereitschaftspotential, which begins some 1s prior to movement. From recordings with subdural electrodes in epileptic patients this is thought to represent activity in both of the supplementary and primary motor areas (Ikeda 1992).

NS2 is a steeper part of the Bereitschaftspotential that is present 300ms prior to movement it is believed that this represents the lateralisation of the brain activity to the motor cortex.

A number of studies on patients with a variety of dystonias have been performed. Unfortunately, the groups that have been studied have comprised different types of dystonia and used differing paradigms. In secondary dystonia, the NS1 and the NS2 have both been found to be reduced in size (Feve 1994) in patients with primary generalised dystonia the NS2 peak amplitude was reduced (Van der Kamp 1995). In writer's cramp studied during movement of the hand, the NS2 component was also found to be reduced (Deuschl 1995). The results are hard to interpret and probably reflect the heterogeneity of the studies.

1.6.7.4. Transcranial electric and magnetic stimulation (TMS)

Transcranial electric and magnetic stimulation has evolved over a number of years to provide a useful technique for the study of brain function. The techniques have become more complex and refined and now allow the function of both excitatory and inhibitory inputs within the cortex to be studied. The investigations into dystonia are reviewed in the following categories.

1.6.7.4.1. Threshold investigations

Basic stimulation of the motor cortex requires a threshold to be found that produces the movement of the appropriate muscle. Investigations into this threshold and into the central conduction time for fast-conducting corticospinal fibres in patients with dystonia found that there was no difference between either of these measures (Thompson 1986).

1.6.4.4.2. Cortical Output Investigations

Further, more detailed studies with magnetic stimulation found more subtle abnormalities in dystonia. The size of EMG responses to magnetic stimulation with increasing background muscle contraction were studied. In general, as background contraction increases, the size of the stimulated EMG increases. The effect in dystonic patients, however, outstrips that in normal subjects (Mavrouidakis 1995, Ikoma 1996). This finding suggests an excess of motor output from the motor cortex during voluntary action in dystonia.

1.6.7.4.3. Cortical Mapping Investigations

Because of the diversity of TMS methodology, it can be used to investigate some spatial abnormalities in dystonia. Investigations mapping cortical sites have shown alteration in the size and location of the cortical hand muscles stimulation sites in patients with writer's cramp compared to controls. This suggests some reorganisation of the motor cortex (Thompson 1996).

Byrnes *et al* (Byrnes 1998) investigated the corticomotor representation of upper limb muscles in focal limb dystonia of differing duration both before and after treatment. They found that in comparison to normal subjects, the dystonic individuals displayed a distorted and displaced corticomotor projection map. Interestingly, they found the

degree of abnormality related to the length of time the patient had had the condition. They also demonstrated some normalisation of these maps after injection with botulinum toxin. In addition to this, they also found similar changes from the clinically unaffected side. They concluded that these reorganisations are slowly evolving and may result from afferent inputs from both affected and unaffected muscles.

2.7.4.4. Silent Period Investigations

Further studies on the excitability of the cortical neurons in dystonia have been performed using TMS. These studies involved active muscle contraction during which a large conditioning stimulus is applied to the scalp. In the control subjects this produces an EMG silent period (Priori 1994). The origins of the silent period remain difficult to elucidate but appear, in part, to be mediated by intracortical inhibition stimulated by the conditioning pulse (Fuhr 1991, Inghilleri 1993, Uncini 1993). It has been shown by a number of studies now that the silent period is reduced in dystonia (Ikoma 1996, Filipovic 1997, Rona 1998) adding weight to the idea that there is a reduction in the inhibition of the motor cortex.

1.6.7.4.5. Sub threshold conditioning stimulation investigations

The reduction in inhibition and increased excitability of the intracortical connections within the motor cortex can be further studied by TMS. This is achieved by giving paired magnetic stimulation's at a variety of interstimulus intervals. In the normal subject, the recorded size of the EMG response in the muscle is both inhibited and facilitated by a prior sub threshold conditioning stimulus, depending on the inter-stimulus interval. A sub threshold conditioning stimulus produced < 6ms prior to the supra threshold test stimulus will produce a reduction in the measured response as

compared to the measure response without a conditioning stimulus. If, however, the inter stimulus time was $> 6\text{ms}$, the measured response was greater than that measured by stimulation alone (i.e. without any conditioning stimulus) (Kujirai 1993). This response is interpreted as being due to inhibitory activity within the intracortical circuits of the motor cortex, and it has also been shown that voluntary movement of the target muscle can decrease the amount of inhibition and facilitation (Ridding 1995a). Ridding *et al*, have studied focal dystonia with this methodology and found that there is a reduction in the inhibition when the patients are tested at rest. This is an interesting finding, again indicating that there is a reduction in the inhibition of the motor cortex (Ridding 1995b).

Edwards *et al*, have used more advanced techniques to examine patients with DYT1 dystonia (MDYT1), as well as non-manifesting carriers of the DYT1 gene (NMDYT1). They found that the MDYT1 group had reduced intracortical inhibition (ICI), shorter silent periods (SP), and absent pre-synaptic phase of reciprocal inhibition. The NMDYT1 group, however, showed similar changes in ICI and SP as MDYT1, but showed no changes in reciprocal inhibition. The authors concluded that dystonia is due to a number of electrophysiological abnormalities, and that the DYT1 gene only predisposes to a subset of these changes, implying additional environmental or genetic subset may be needed to manifest the dystonia (Edwards 2003).

1.6.7.4.6. Supra threshold stimulation studies

In other experiments using double stimulus methods, Chen *et al* didn't give a conditioning stimulus but instead two supra threshold stimuli over the motor cortex at varying interstimulus intervals. In normal subjects at rest this produces a suppression of the response to the second stimulus of $>200\text{ms}$ and a suppression of 130ms during

voluntary activity. In patients with focal dystonia the suppression was normal at rest but was significantly reduced during voluntary contraction of the target muscle. Again this indicates that there is an abnormally low amount of inhibition of the motor cortex (Chen 1997). Others have failed to reproduce this results (Rona 1998).

Sommer *et al*, by using a modification of this type of paradigm in which the centre of stimulation was moved away from the hand cortical representation, described a number of findings in dystonic patients. They demonstrated both reduced intracortical inhibition and increased intracortical facilitation in surrounding motor areas to cortical representation of the hand area in dystonic patients. Interestingly, they showed similar changes in the hand area in both patients with blepharospasm as well as focal hand dystonia (Sommer 2002).

1.6.7.4.7. Repetitive TMS (Sub and Supra threshold)

Repetitive chains of low frequency supra threshold stimuli have also been shown to induce increased motor evoked potentials (in the first dorsal interosseous muscle) in patients with writer's cramp (Siebner 1999). This abnormal facilitation of the motor output during continuous stimulation of the motor cortex may help to explain the onset of cramping during movements.

In a further experiment Siebner *et al*, studied the effect of sub threshold rTMS to the premotor cortex on regional cerebral blood flow (rCBF) in patients with focal dystonia. Interestingly, rTMS in patients produced greater reductions (premotor areas, putamen, thalamus) and enhancements (cerebellum) in rCBF than those seen in controls. This effect was not due to motor performance and hence, it was concluded, that this increased responsiveness of the motor system to rTMS maybe a physiological trait that characterises dystonia (Siebner 2003).

1.6.7.4.8. TMS with peripheral stimulation

Using peripheral stimulation of the median nerve in conjunction with TMS and motor evoked potential (MEP) recordings, Abbruzzese *et al* demonstrated that at certain intervals (200ms), peripheral stimulation significantly reduced the MEP in control subjects and patients with torticollis. In studying patients with writer's cramp however, it was shown that there was a significant facilitation of MEP size in writer's cramp (Abbruzzese 2001). They concluded that this demonstrated central processing of sensory inputs in dystonia was abnormal and this contributes to the abnormal motor output.

1.6.7.5. Magnetoencephalographic (MEG) studies

MEG measures the intercellular currents of the neurons (as magnetic dipoles) in the brain giving direct information on the brain's activity, either spontaneously or to a given stimulus. In a study of patients with occupational hand cramps performed by stimulating individual digits and mapping their cortical representations in the sensory cortex, the medial-lateral extent of the territory covered by these representations was calculated and found to be reduced in the dystonic individuals (Elbert 1998). The authors concluded that this fusion of sensory information is important in misguiding the motor output.

1.6.7.6. In Vivo Neurophysiological Studies

Dystonia that is refractive to medical therapies can be treated surgically. Initially thalamotomy was used to some benefit (Cooper 1976, Andrew 1983, Tasker 1988, Cardoso 1995). More recently techniques have become more refined and pallidotomy

has been conducted both unilaterally and to greater benefit bilaterally (Iacono 1996, Lozano 1997, Lin 1998, Ondo 1998, Vitek 1998, Lai 1999). Presently interest lies with pallidal deep brain stimulation (DBS) as it may be more effective and is thought not to cause permanent brain damage. It has been performed successfully in generalised (Coubes 2000, Tronnier 2000, Kumar 1999) and focal dystonias (Krauss 1999, Islegel 1999, Andaluz 2001). It is interesting to note that the improvements accrued by DBS may take months to fully develop.

This shift to surgical treatment in intractable dystonia provides an opportunity to study dystonic individuals directly and hence give greater insight into pathophysiological abnormalities. Recordings from the internal segment of the globus pallidus have shown a decrease in the discharge rate of neurons (Ondo 1998, Lozano 1997 Vitek 1998). In addition to this the pattern in which the GPi neurons fire is also abnormal, showing irregular grouped asynchronous discharges. However, it remains unclear, as to whether some of these changes relate to possible effects of the anaesthetic agents or not (Vitek 2002).

Further investigations at the time of surgery have demonstrated abnormalities at the level of the thalamus. Lenz *et al* studied the ventral caudal nucleus of the thalamus (Vc) in patients with dystonia during somatic sensory stimulation and compared them to patients with essential tremor. They showed that there was significant somatotopic reorganisation of the receptive fields and a disorganisation of the projection fields in dystonic subjects (Lenz 1999). Whether this change was causative or secondary to the dystonia was not speculated.

1.6.8. Functional Imaging Studies

1.6.8.1. Positron Emission tomography (PET)

PET imaging is a method of functional imaging that relies on changes in regional blood flow (RBF). PET produces images of the brain by detecting the radiation emitted from radioactive substances. These substances are injected and are usually tagged with a radioactive atom that has a short decay time. The PET camera detects the gamma rays given off at the site where a positron emitted from the radioactive substance collides with an electron in the tissue.

1.6.8.1.1. Regional Blood Flow

The first interesting information to come from PET imaging came from a study by Tempel and Perlmutter in 1990 when they examined the RBF change in patients with dystonia during vibration of the finger pads. They found that during such stimulation there was a significant decrease in the blood flow to the sensorimotor cortex in patients with focal dystonia (Tempel 1990). They also noted that this was present whether the affected or unaffected side was stimulated. In further studies (Tempel 1993) they described a significant reduction in the supplementary motor area (SMA) regional blood flow again independent of which side (symptomatic or asymptomatic) they stimulated.

Ceballos-Baumann *et al*, in a series of PET experiments, defined a number of regional blood flow abnormalities in generalised and focal dystonia during paced movements. Firstly, this group demonstrated an overactivity of the prefrontal motor planning areas and underactivity of the execution areas during movement (Ceballos-Baumann 1995). Further work by the same group concentrating on writer's cramp both before and after receiving botulinum toxin therapy, showed an overactivity in the ipsilateral premotor cortex, the insula areas of the parietal cortex, and cerebellar vermis compared to the control group. Underactivation compared to controls was noted in the contralateral

motor cortex, caudal SMA, anterior cingulate gyrus, mesial parietal and thalamus. Treatment with botulinum toxin increased parietal activation still further, but had no effect upon the motor cortical activity (Ceballos-Baumann 1997).

Further studies utilising PET estimates of regional blood flow and with more complex paradigms have shown significant decreases in RBF during sustained contraction and writing tasks in the contralateral sensorimotor and premotor cortices (Ibañez 1999). Other studies in patients with familial generalised dystonia have shown similar results except for an increase activity in the SMA activation (Playford 1998).

1.6.8.1.2. Metabolic studies

PET has been widely used to study potential metabolic abnormalities in dystonia. Resting glucose metabolism has been assessed by a number of investigators and has found to be increased (Chase 1988) and normal (Otsuka 1992) in contralateral striatum and decreased in the caudate, lentiform nuclei and the frontal projections field of the mediodorsal thalamic nucleus (Karbe 1992).

Further work to try and clarify the issues was undertaken in patients with torticollis; both studies reported an increase in glucose metabolism in the lentiform nuclei (Galardi 1996, Magyar-Lehmann 1997).

Eidelberg *et al*, using a principal components method, described putaminal hypermetabolism and later linked this to thalamic hypometabolism. It was postulated that these results argued for there being an increase in the activity of the direct striato-pallidal pathway in dystonia. This overactivity would cause inhibition of the globus pallidus and hence reduced output to the thalamus (Eidelberg 1995).

Further work on glucose metabolism at rest by the same group was performed in patients with primary generalised dystonia and asymptomatic carriers of the DYT1

gene (Eidelberg 1998) and more recently in others dystonia genotypes (Trošt 2002) and blepharospasm (Hutchinson 2000). Interestingly, these studies showed increased metabolism in all of groups in three areas: the lentiform nucleus, the cerebellum and the supplementary motor cortex. These findings are most interesting in that it is clear that some other factor in addition to possession of the gene is at work in dystonia. These factors could be environmental, behavioural or genetic.

1.6.9. Peripheral studies of sensation

1.6.9.1. Sensory discrimination in dystonia

The ability of patients with focal dystonia to perceive sensation is apparently quite normal. More subtle abnormalities however, have been described. Patients with focal dystonia have been studied during simple spatial (Tinazzi 1999b, Bara-Jimenez 2000a) and temporal discrimination tasks (Tinazzi 1999b, Bara-Jimenez 2000b). Compared to the control subjects, patients with dystonia showed no difference in spatial discrimination. There was a difference in the ability of the patients to discriminate temporally between two separate stimuli. The threshold (measured in milliseconds between stimuli) for detection was higher in the dystonic group and had a correlation with length of symptoms. It was speculated that this abnormality contributes to impaired sensorimotor integration and hence impaired motor output (Bara-Jimenez 2000a).

1.6.9.2. Complex sensory processing in dystonia

Using specific frequency vibration to activate muscle spinal Ia afferents during a sensory tracking test, investigators have demonstrated that dystonic subjects display an abnormal perception of motion but a normal sense of position (Grunewald 1997).

These findings are widespread in dystonic individuals and are not confined to symptomatic areas. This has been interpreted as suggesting that the processing of information from the muscle spinal afferents is abnormal in dystonia. Other studies have found similar findings that can be reversed by anaesthetic block (Kaji 1995b). Further studies of sensation in affected individuals have demonstrated impairment in graphesthesia and stereognosis (Byl 1996). Slowness of dystonic patients to switch between motor tasks (Agustino 1992) has also been interpreted as an abnormality associated with the sensory organisation of complex movements.

1.7. Summary of Literature

Combining all the different methodologies used to study dystonia is difficult, as is assessing the pathological boundaries between generalised and focal dystonia. It is best therefore to consider the cumulative findings from a number of standpoints.

1.7.1. Motor (output) abnormalities

There is good evidence for abnormal motor cortical activity during the execution of tasks. These abnormalities are present in structures involved in both the preparation and execution of the task. The evidence for functional abnormalities at rest is less convincing. There appears to be good evidence for the lack of inhibition and indeed excessive facilitation within the motor cortex itself. There is also some evidence for an abnormal somatotopic organisation of the motor cortex.

1.7.2. Sensory (input) abnormalities

There are clearly a number of abnormalities relating to complex sensation; there is a particular abnormal 'setting' of the sensory system to detect movement (motion). In addition to this there are clear abnormalities in the processing of sensory information. Abnormal organisation of the sensory receptive field at a thalamic and perhaps a

cortical level has been demonstrated. Evidence for abnormal activation of cortical sensory areas also exists, but some of this evidence is contradictory.

1.7.3. Sensorimotor (integration) abnormalities

The integration of sensory information into a motor plan has been shown to be abnormal in dystonia. Exact detail as to how the integration is abnormal doesn't exist. It is also unclear where this integration takes place.

1.7.4. Site of abnormalities

The main seat of abnormalities in dystonia appears to be either in the basal ganglia, the cerebral cortex or in both. Other abnormalities described at the peripheral nerve and spinal levels could be explained by alterations in the descending influences from the brain.

There is conflicting evidence regarding the generalisation of abnormalities in patients with focal dystonia. Some studies suggest an underlying bilateral and widespread abnormality whilst others refute this.

1.8. Research Project Development

At the time the research was planned, no functional Magnetic Resonance Imaging (fMRI) studies in dystonia were in print. The main objectives of the research were to look into some of the issues raised above. With this in mind, the research was planned to consist of four studies, each looking into slightly different aspects of movement planning and execution as well as the sensory and motor organisation and integration during tasks.

Chapter Two

FMRI Experiments: NMR, MRI, Hardware, Image Processing & Analysis

2.1. Nuclear Magnetic Resonance - Basic Principles

It was essential for the completion of the projects described in the following chapters to undertake the scanning of the patients on my own. The MRI scanner used for the experiments was a custom built dedicated 3.0 Tesla research machine on an in-house platform and the operation of it was complex compared to clinical scanners. It was imperative therefore that a working knowledge of the physical principles of magnetic resonance was acquired.

2.1.1. Introduction to NMR

Magnetic resonance imaging (MRI) was borne out of work on Nuclear Magnetic Resonance (NMR) reported independently by two groups of scientists in the 1940's. They observed that transitions could be induced between magnetic spin levels of certain nuclei and that these transitions corresponded to frequencies in the radiofrequency range (Purcell 1946, Bloch 1946). Over the next few decades, rapid

progress was made in the field of NMR physics with the main emphasis being placed on spectroscopy. NMR became one of the standard method of determining the structure of organic compounds.

In the early 1970's, the first system of NMR imaging was developed (Lauterbur 1973). In the next five years, the first live human patients where scanned (Damadian 1977) following which the rapid evolution of MRI as an essential clinical tool began.

2.1.2. The precessing proton

Although there are a number of naturally abundant elements with nuclear spin, for the purposes of this discussion the hydrogen atom will be considered, as it forms the basis for MRI. This due to its high natural abundance and concentration, approximately 55% of the human body is composed of water with soft tissues and blood containing 80% water. The nucleus of the hydrogen atom is positively charged and it possesses spin. The spinning motion induces a local magnetic field, so that in effect it is behaving like a tiny bar magnet (Figure 2.1). Within any tissue, the magnetic fields of these protons are randomly orientated and hence there is no net magnetism.

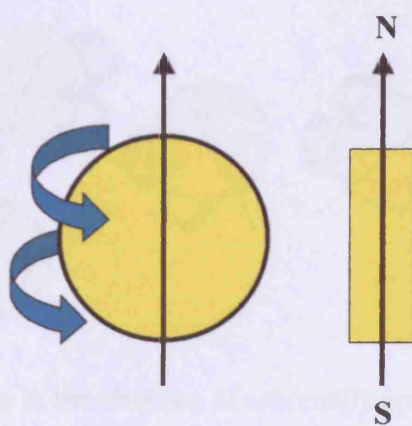


Figure 2.1. Precession of proton and resultant induced magnetic field.

2.1.3. Effects of External Magnetic Field

When an external magnetic field, B_0 , is applied to this system, the individual atoms arrange themselves in the magnetic field with either a parallel or an anti-parallel orientation (Figure 2.2a & Figure 2.2b). This external magnetic field generates a torque on the spinning protons 'spins', so instead of lining up directly with the field, they revolve or precesses around the magnetic field direction (Figure 2.3). The frequency at which they precess is proportional to the external magnetic field (B_0) applied. The anti-parallel state has a slightly higher energy, so a fraction more of the protons line up in the parallel state. Thus, a resultant net nuclei magnetisation will be in the direction of the externally applied magnetic field. This is termed longitudinal magnetisation (Figure 2.4), and it is this small difference in the number of spins between the two energy states that the signals generated in MRI are based on.

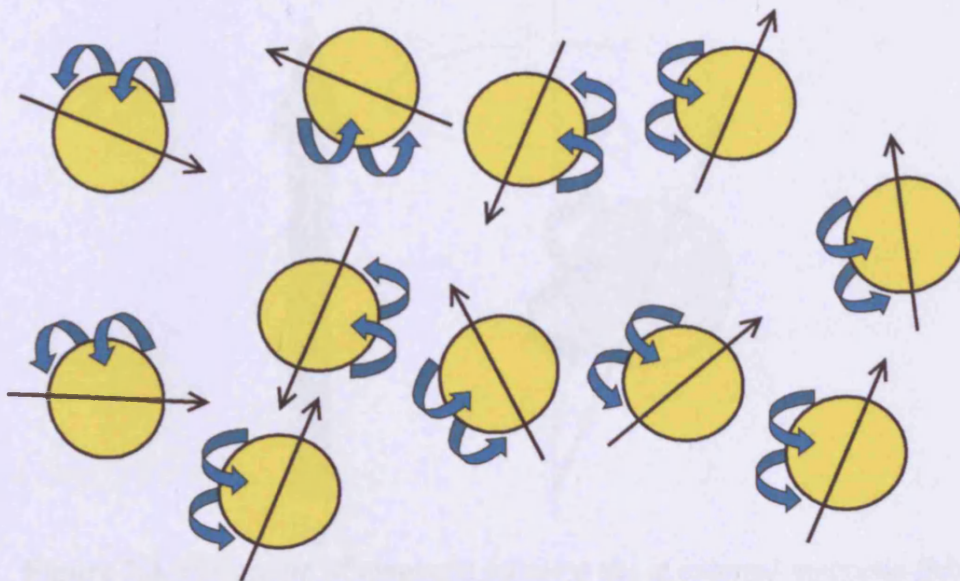


Figure 2.2a. Protons in the absence of externally applied magnetic field

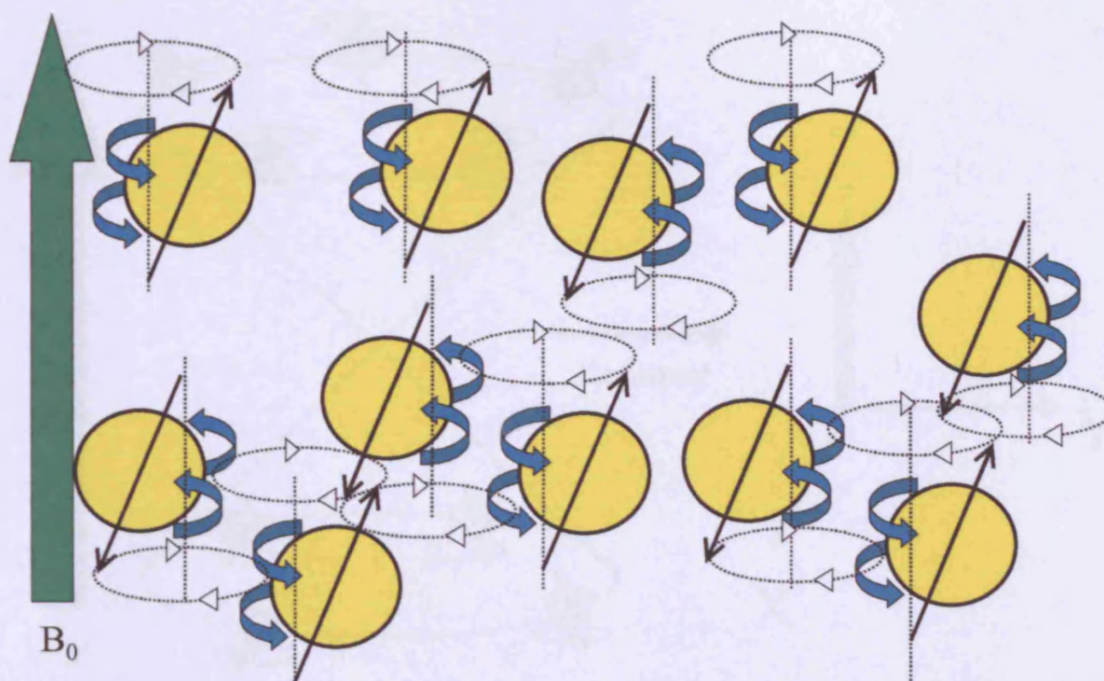


Figure 2.2b. Protons in presence of externally applied magnetic field

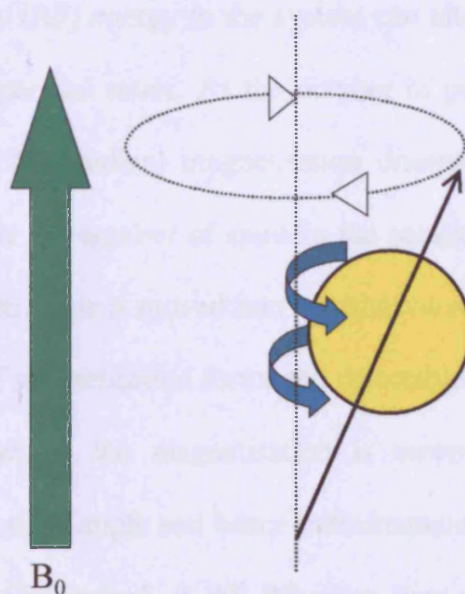


Figure 2.3. Precession of magnetic moment about external magnetic field

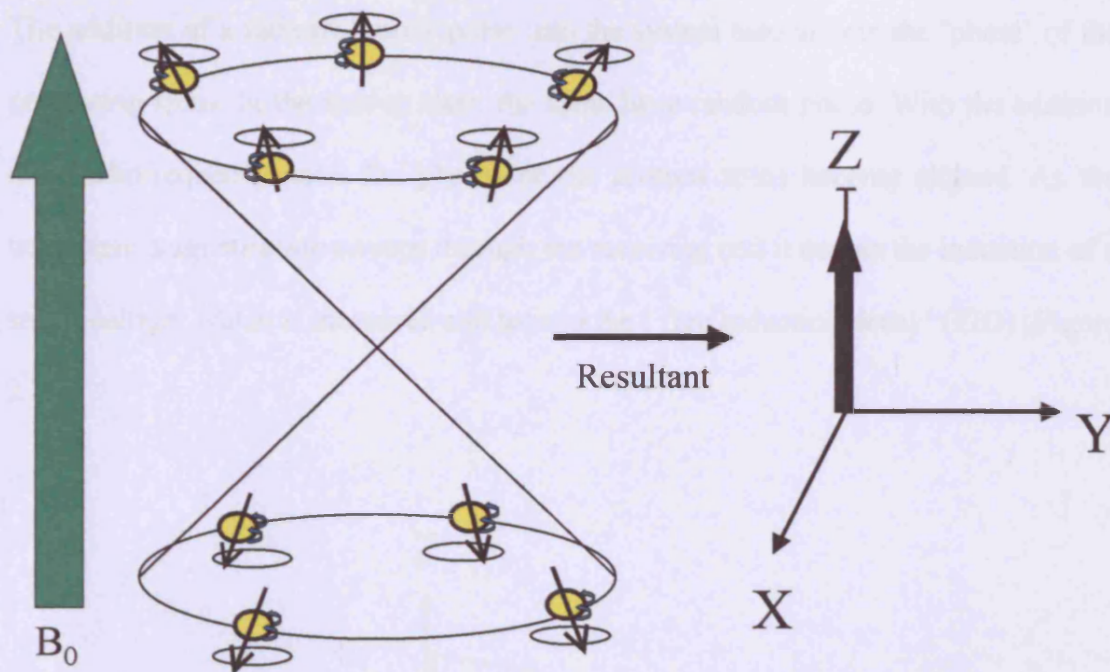


Figure 2.4. Resultant magnetisation in direction of external magnetic field

2.1.3. Effects of radiofrequency field

Applying radiofrequency (RF) energy to the system can alter the proportion of spins in the parallel and anti-parallel states. As the number of protons in the anti-parallel state increases, the net longitudinal magnetisation dissipates. If the RF energy is applied long enough, and the number of spins in the parallel and antiparallel state is equalised, the net magnetisation is moved into the transverse plane. (Figure 2.5.). The transverse component of magnetisation forms the detectable and measurable signal in MRI. The degree to which the magnetisation is moved from the longitudinal equilibrium is measured as an angle and hence radiofrequency pulses are described as in terms of degrees or 'flip angles'. A 90° RF pulse flips all magnetisation into the transverse plane whilst a RF pulse of 180° will totally 'invert' the longitudinal magnetisation, inverting the population of spins in the parallel and antiparallel states, without producing any transverse (measurable) magnetisation (Figure 2.6.).

The addition of a radiofrequency pulse into the system also affects the 'phase' of the precessing spins. In the resting state, the spins have random phase. With the addition of a radiofrequency pulse the phases of the protons spins become aligned. As the transverse magnetisation sweeps through the receiving coil it causes the induction of a small voltage, which is measured and termed the ' free induction decay' (FID) (Figure 2.7).

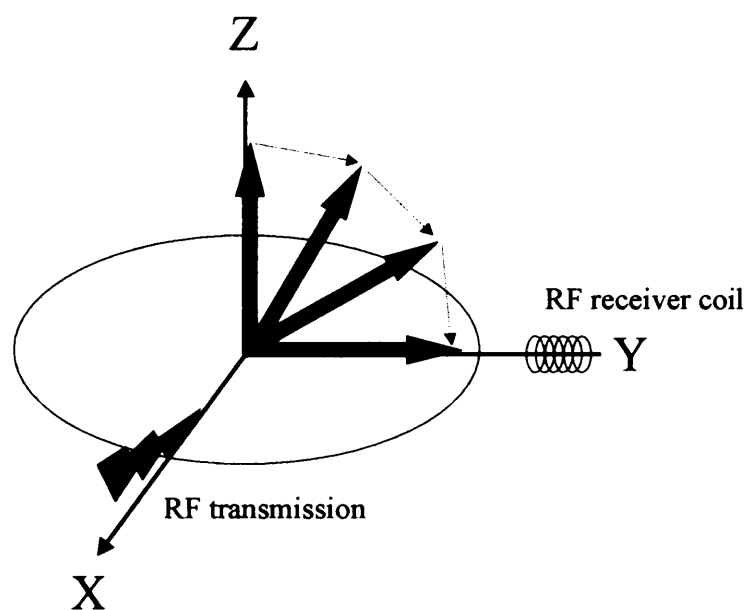


Figure 2.5. Application of a 90° pulse to flip all longitudinal magnetisation into the transverse plane

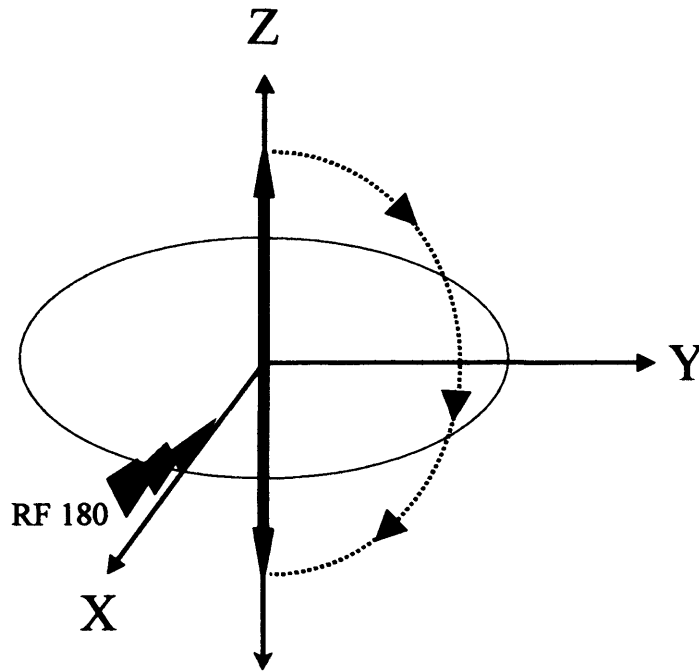


Figure 2.6. Application of a 180° pulse to invert longitudinal magnetisation

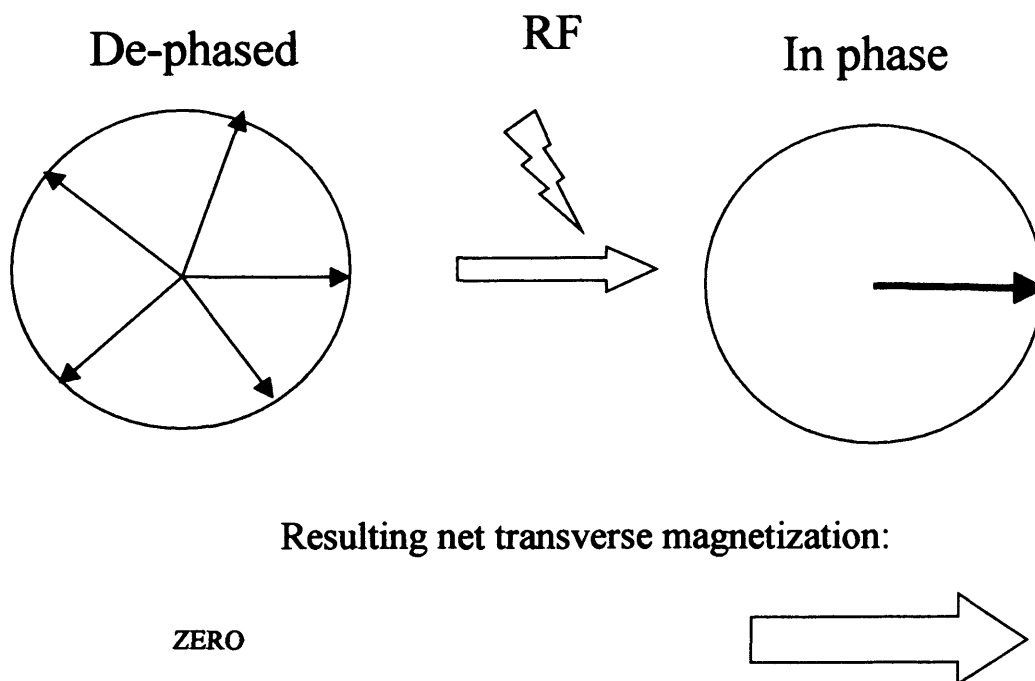


Figure 2.7. Effect of 90° pulse on the phase in transverse plane

2.1.4. Relaxation Processes

Once the RF pulse is switched off, the nuclei will relax to their original equilibrium state. During this process the 'excited' spins give the energy gained from the applied

RF pulse up, and the transverse magnetisation decays back to zero. The nuclei release energy in two ways: 1) energy is given up to neighbouring molecules in the environment, so-called 'Spin-Lattice' or T_1 relaxation 2) the energy is given up to other nearby molecules, so-called 'Spin-Spin' or T_2 relaxation.

2.1.4.1. T_1 (Spin-Lattice relaxation)

T_1 is a time constant and represents the recovery of the longitudinal magnetisation. This is an exponential process in which 63 % of the longitudinal magnetisation recovers in T_1 milliseconds. The surrounding 'lattice' has thermal energy which creates small fluctuating magnetic fields that allow the energy to be transferred from the proton and dissipated as heat (Figure 2.8). This transfer of energy is dependent on the tissue type (e.g. fat and CSF) and the external magnetic field strength.

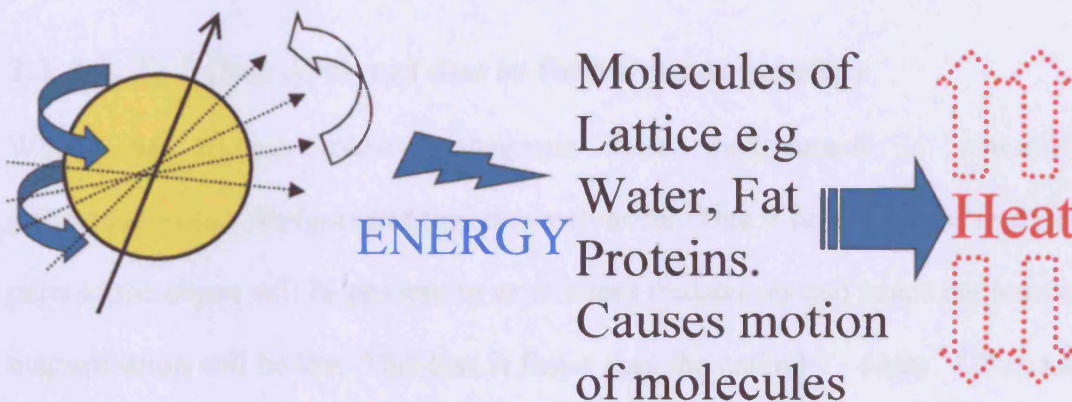


Figure 2.8. Transfer of energy to lattice

2.1.4.2. T_2 (Spin-Spin Relaxation)

T_2 is the time constant concerned with the loss of transverse magnetisation (decay of the FID signal). This is again an exponential process. It is important to note that the

loss of transverse magnetisation occurs at a different rate to the recovery of longitudinal magnetisation. During the relaxation process, the spinning proton gives up its energy not only to the lattice but also to non-excited proton that causes it in turn to become excited. Equilibrium of excited and non-excited protons is thereby established. However, as the energy is transferred, the spins of the protons become 'de-phased' and hence transverse magnetisation is lost (Figure 2.9). It is this extra mechanism of 'dephasing' that causes T_2 to be less than T_1 .

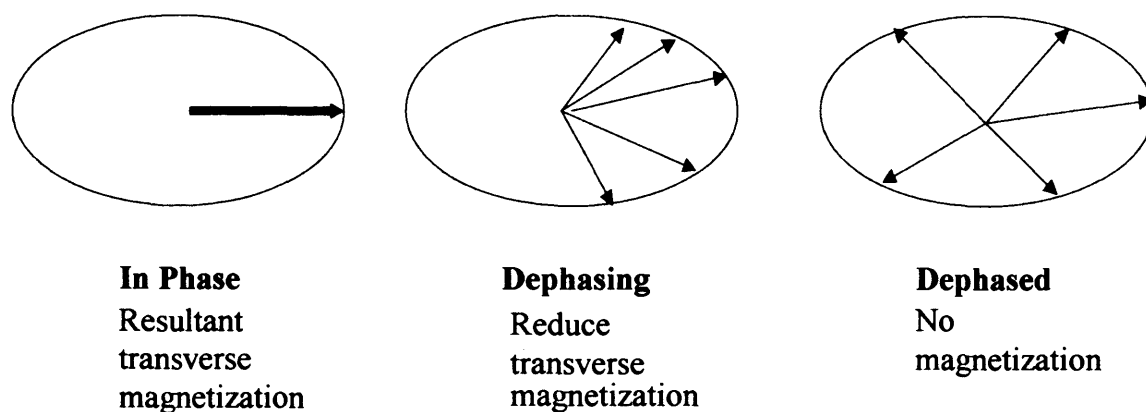


Figure 2.9. Dephasing of transverse magnetisation following 90° pulse

2.1.4.3. T_2^* (loss of signal due to field inhomogeneity)

When the applied external magnetic field environment is non-uniform (inhomogeneous), dephasing of the spins will occur. This is because spins in different parts of the object will be precessing at different frequencies and hence net transverse magnetisation will be lost. This loss is faster than the natural T_2 decay. T_2^* is a time constant that reflects the loss of magnetisation due to natural T_2 processes as well as technical imperfections of the applied external magnetic field and any magnetic field inhomogeneity due to the object (being scanned) itself, such as sinuses in the brain.

2.1.4.4. Free Induction decay

In a simple MRI experiment, following a 90° RF pulse the transverse signal amplitude begins to decay with a time constant T_2^* . This signal decay is called free induction decay (FID) (Figure 2.10). Free induction decay reflects transverse magnetisation from the object being scanned.

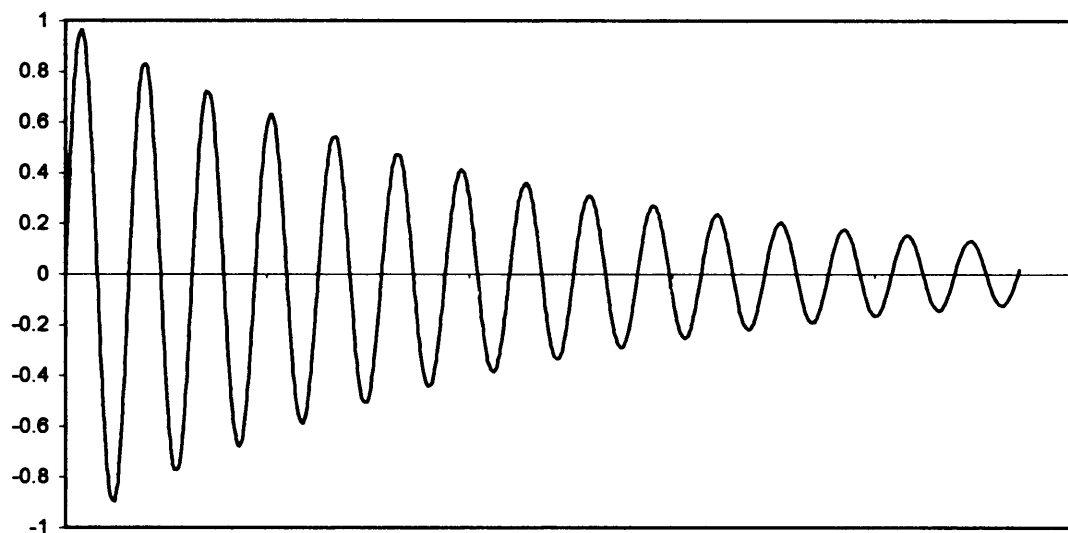


Figure 2.10. Free Induction Decay of an 'off-resonant' signal

2.1.5. Image Formation

Image formation in MRI requires accurate localisation of MRI signals. The assignment of these signals in space is called 'spatial encoding'. This is achieved by the application of linear magnetic field gradients. These are graduated field offsets that are added or subtracted to the main external field. Within the MRI system there are three gradients orientated in the three orthogonal directions. The application of a gradient will cause each strip of the object in the specified gradient direction to be affected. Application of all three gradients during the imaging sequence allows '3-D magnetic identification' of the object being scanned. To spatially encode an imaging plane, slice selection is used, whilst to encode in plane, frequency and phase encoding are employed. Sections 2.1.5.2 & 2.1.5.3 outline spatial encoding for 2D Fourier

imaging the basic technique for anatomical imaging. Section 2.3.1 describes spatial encoding using EPI, the technique for rapid imaging employed in fMRI.

2.1.5.1. Slice selection

To define a slice (plane) of the object, a linear (Z) gradient is applied in the presence of a RF pulse. The gradient axis responsible for slice selection determines the image plane acquired e.g. transaxial, coronal or sagittal. As the linear gradient is applied, the resonance frequency across the object is altered; for example, we can make the resonant frequency lower at the feet and gradually increase in strength to a maximum at the head. The location of the slice we image along this gradient is determined by frequency at which we apply the RF pulse. The strength of the slice select gradient and the frequency of the RF pulse therefore determine the excitation plane. Slice thickness is determined by controlling the range of frequencies in the RF pulse called the 'bandwidth'. To acquire a thinner slice, a narrower RF pulse bandwidth can be used or the slope of the magnetic field increased. Usually, to acquire several slices, several RF pulses of a given bandwidth are applied across a range of frequencies with the slice select gradient amplitude held constant.

2.1.5.2. Frequency encoding

This is achieved by the application of a linear (X) gradient. Because the gradient adds (or subtracts) to the main external field, it has the effect of causing each strip along the gradient to have a signal at a slightly different precessional frequency. Thus the object is said to be frequency encoded along the direction of the gradient. Given that we know the strength and direction of the applied gradient, every frequency component of the measured signal can therefore be assigned to a specific column of the object being imaged. (Figure 2.11).

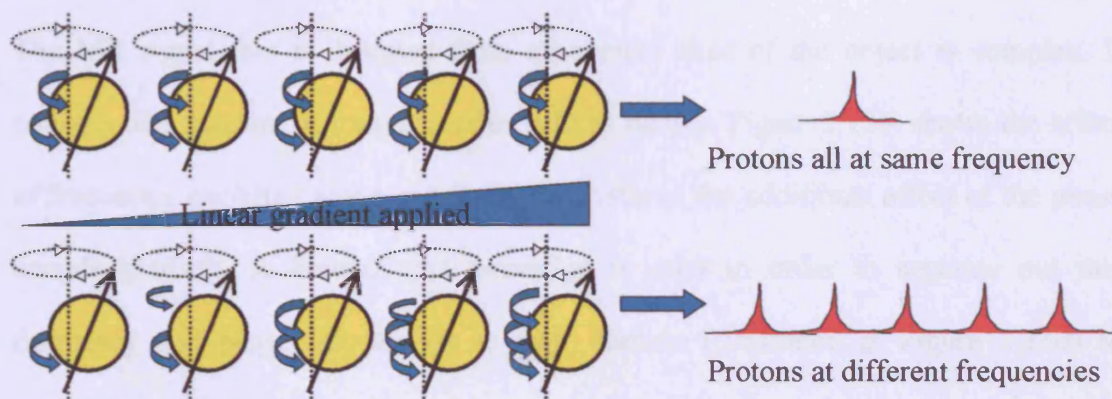


Figure 2.11. Effect of applying a linear frequency-encoding gradient to alter the precession frequency of protons in each column. Note the number of arrows reflects the rate of precession of the spins.

2.1.5.3. Phase encoding

Whilst the frequency encoding gradient is held constant in one direction, another (Y) gradient is applied. This gradient has the effect of altering the phase of the individual spins with the plane (Figure 2.12). This gradient is applied for each projection but the strength of it varied. By this mechanism each 'row' of the image is encoded.

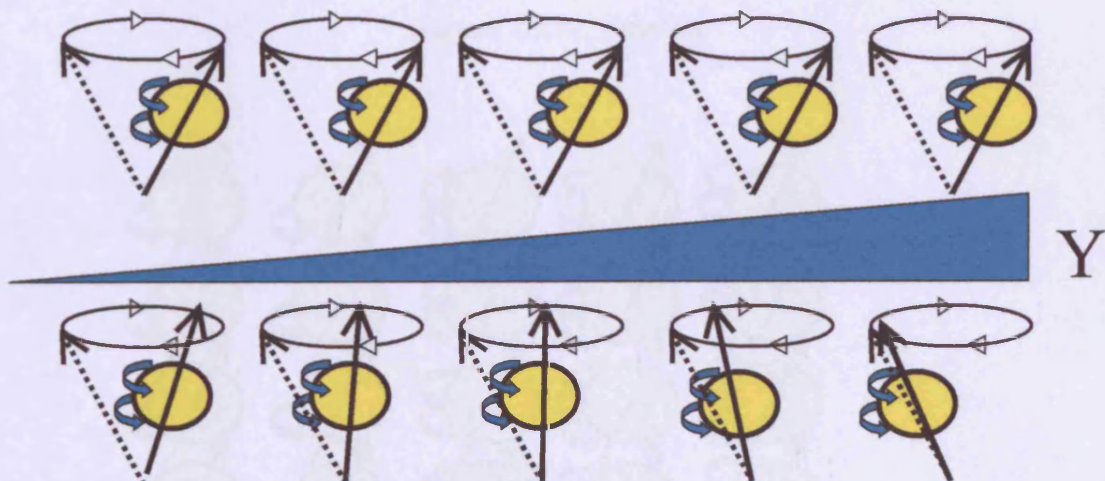


Figure 2.12. Effect of applying a linear phase encoding gradient to alter phase of protons in each row

2.1.5.4. Fourier transformation

The MR signal that is detected from an excited slice of the object is complex. It consists of a mixture of frequencies as well as phases. Figure 2.13b. shows the effect of frequency encoding alone and figure 2.13c shows the additional effect of the phase encode gradient. A Fourier transformation is used in order to separate out this frequency and phase information in time domain (illustrated in Figure 2.13d) to reconstruct an image.

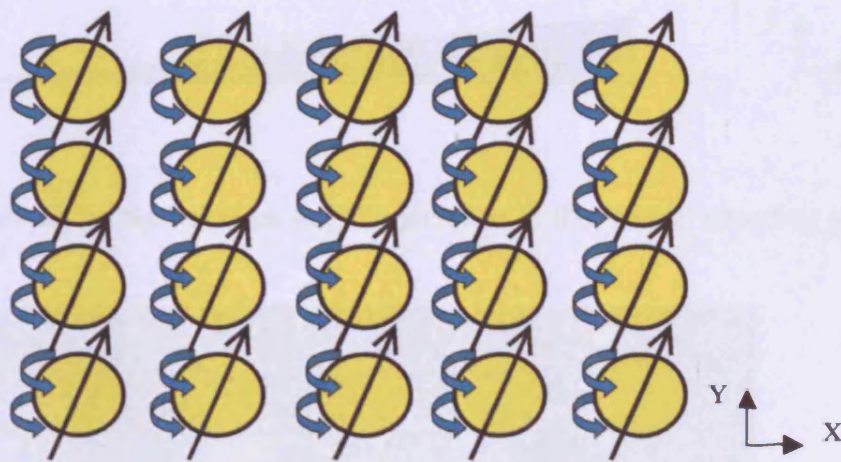


Figure 2.13a. Baseline

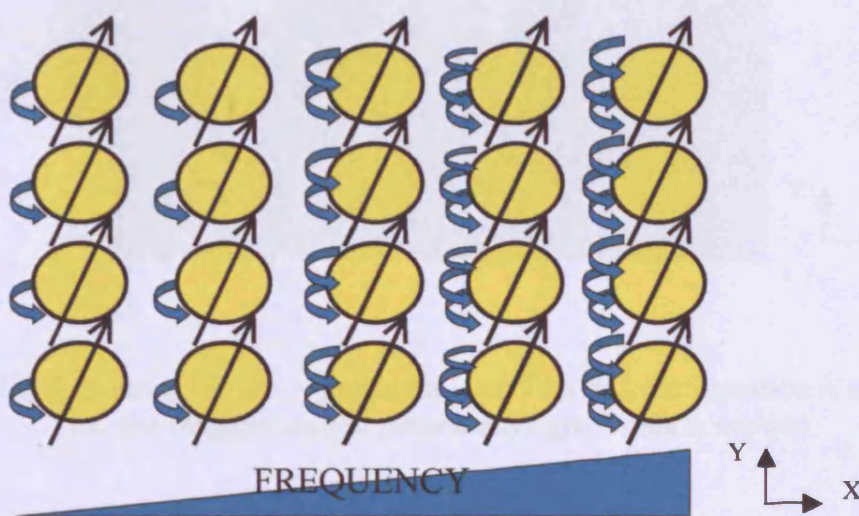


Figure 2.13b. Spatial Encoding: Application of 'the frequency' encoding gradient

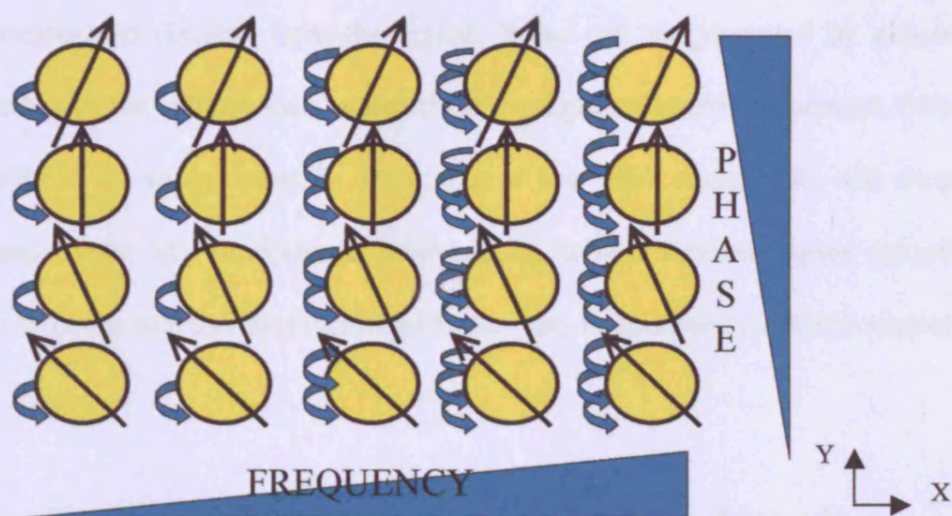


Figure 2.13c. Spatial Encoding: Application of the 'phase' encoding gradient

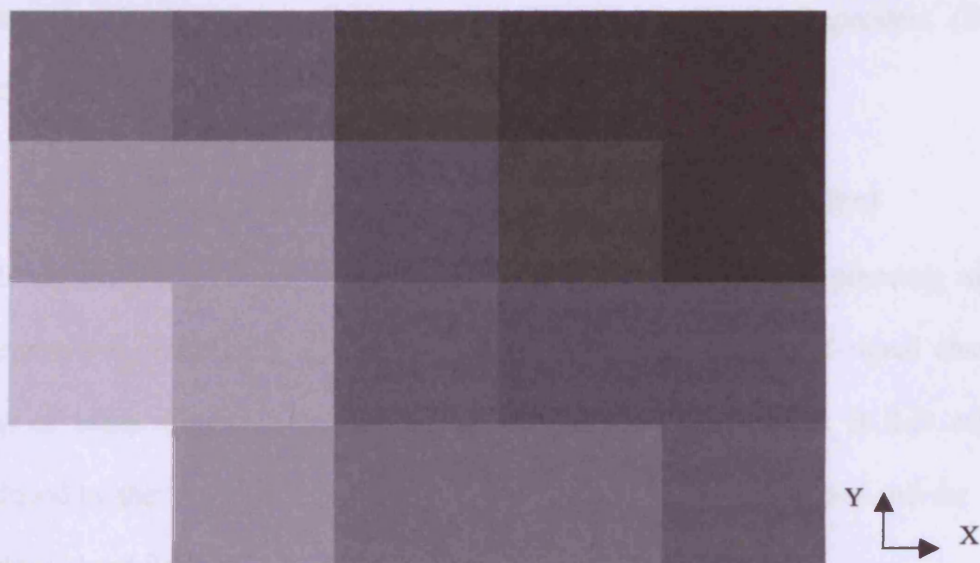


Figure 2.13d. After selection of particular slice Fourier transformation is used to sort out the frequencies and phases and a grey scale is applied.

2.1.6. Signal to Noise Ratio

The signal to noise ratio is an important parameter to understand. Signal is the product of the experiment and increases with field strength; noise interferes with the experiment and detracts from the signal. Noise can be generated by physiological properties of the subject such as heartbeat, background radio frequencies, (which can be reduced by radiofrequency screening of the MRI equipment) and frequencies intrinsic to the MR equipment. Additionally, subject motion causes smoothing of adjacent pixels together and increased noise. This is discussed further in section 2.6.4.

2.2. Functional Magnetic Resonance Imaging

There are now a number of ways to perform functional magnetic imaging. The studies described in this thesis (presented in chapters three to six) all used a similar technique of functional imaging employing Blood Oxygenation Level Dependent (BOLD) contrast.

2.2.1. Blood Oxygenation-Level Dependant (BOLD) Effect

Understanding the biological nature of this effect is key to the planning and the interpretation of the studies. The BOLD effect allows detection of signal change in areas of brain that have become more metabolically active. The BOLD effect is produced by the interaction between the oxygenation state of the blood and the region cerebral blood flow and blood volume to active areas of the brain.

2.2.1.1. Oxyhaemoglobin and deoxyhaemoglobin

The key feature of haemoglobin that allows it to act as a biological contrast agent in fMRI is ability to bind and dissociate from oxygen, changing its magnetic properties. Deoxyhaemoglobin, because of its unpaired electrons, has its haeme moiety in a high

spin state (2+). In this state, the molecule is paramagnetic. When oxygen is bound to haemoglobin, one of the free electrons is transferred to the oxygen molecule and the haeme changes to a low spin state (3+). This molecule has no paramagnetic effect and is termed diamagnetic.

Because of its paramagnetic effect, deoxyhaemoglobin in red blood cells causes changes in the magnetic susceptibility in the surrounding tissues, and this results in dephasing of the precessing spins and hence shortening of T_2^* and loss of signal within and around blood vessels containing deoxyhaemoglobin.

2.2.1.2. Regional Cerebral Blood Flow

PET studies (Fox 1986, Fox 1988) have shown that there is a large increase in regional cerebral blood flow to brain regions that are activated by tasks. Critically, they have also shown that there is only a small increase in oxygen consumption following the increase in neuronal activity. From these findings, it was concluded (Ogawa 1990) that the flow of blood into an activated brain region significantly exceeds the oxygen consumption demands of that area. This means that on brain activity, the levels of de-oxyhaemoglobin are reduced, T_2^* is increased and hence an increase in the intensity of the T_2^* weighted images results.

This increase in the intensity of MR images on brain activity is termed the BOLD effect, and can be measured as the percentage signal intensity change in a specific brain region.

2.2.1.3. BOLD Haemodynamic Effects

Clearly it takes only milliseconds for brain areas to become activated and start demanding greater blood flow. However, due to brain vasculature the cerebral blood flow does not change instantaneously and there is always a delay from the execution

of a task to the maximum BOLD change. This peak in the BOLD signal change typically occurs 4-6seconds after the onset of task performance (Blamrie 1992).

2.3. Sequence

2.3.1. Echo Planar Imaging

The fMRI studies outlined in this thesis all use the Echo Planar Imaging (EPI) acquisition sequence. EPI is an ultra fast scanning technique (Mansfield 1977). The speed of this technique comes from the fact that all the data for the image is acquired after a single RF excitation pulse. After the simultaneous application of an excitation pulse and the slice select gradient, a series of echoes with different phase encoding are created. A constant X gradient is applied while the Y gradient is rapidly switched in order to repetitively refocus the echo signals. Because of the different timing of the echoes, each sample will represent a different line of Fourier (k) space.

There are many variants of EPI; the one that was used for the studies in this thesis is called MBEST (Modulus Blipped Echo-planar Single-pulse Technique (Ordidge 1988). Figure 2.15 shows the EPI pulse sequence.

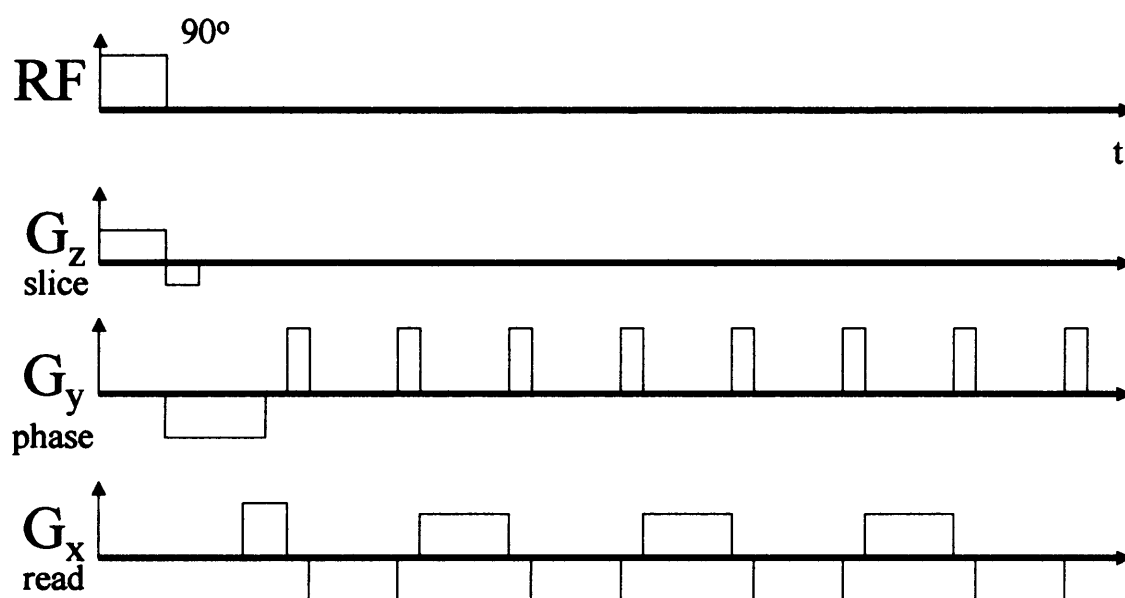


Figure 2.15. The MBEST EPI sequence

2.4. Functional Imaging Hardware

All of the imaging for the studies presented in this thesis took place at the Magnetic Resonance Centre in the School of Physics and Astronomy at the University of Nottingham. The MRI scanner used was a 3.0 Tesla system that was custom-built and maintained by the department. The fMRI set up consisted of a number of hardware components that were utilised throughout the period of investigation.

2.4.1. The External Magnetic Field

A large 3.0 Tesla horizontal bore superconducting magnet was used. The internal windings of the magnet (Oxford Magnet Technologies) were constantly immersed in liquid helium surrounded by an outer ‘pocket’ of liquid nitrogen. This was essential to maintain the superconductance of the magnet. The magnetic field generated by the system is temporally extremely stable; this is essential for ongoing MRI experimentation.

2.4.2. Shims

As soon as an object is placed in the magnet it affects the magnetic field homogeneity, and hence corrections are needed to restore this. Shimming essentially allows minute adjustments to the magnetic field to ensure homogeneity. A degree of passive shimming is built into the magnet structure, and in addition to this it is important to actively shim. This was accomplished via manually shimming using a system of passing current through the shim coils to produce magnetic fields that can be adjusted to smooth out field variations. This procedure was undertaken prior to scanning each subject.

2.4.3. Gradient Coils

The gradient coil used in all the experiments was built locally in the department. Gradient coils provide the linear magnetic field variation along the X, Y, and Z-axes that enables the spatial encoding of the MR signal (described in 2.1). The three linear gradients are fed from Techtron linear power amplifiers. When using EPI, the readout gradients are switched extremely rapidly (from positive to negative) in order to achieve the rapid scanning. In this thesis, a switching frequency of 1.9KHz was used for BOLD studies (sagittal and coronal orientation).

2.4.4. Radiofrequency Transmission and Receive Systems

The design of the coils used was such that they both transmitted the RF pulse as well as received the MR signal. In the studies conducted in this thesis, two RF coil types were used.

2.4.4.1. Whole Head coil

A volume head coil was used in chapters 3 and 4, which allowed coverage of the whole of the head. This coil was a Birdcage design, which was manufactured within the department. The design consisted of a number of wires running in the Z – direction, arranged to give a cosine current variation around the circumference of the coil.

2.4.4.2. Surface coil

Surface coils can be designed to improve investigation of specific areas of the brain, for example the occipital cortex or the motor and sensory cortex on one side of the

head. These coils yield high signal to noise ratio thereby facilitating more detailed higher resolution imaging. This type of coil was employed in chapters 5 and 6.

2.4.5. Equipment for Functional Experiments

2.4.5.1. Inverted Glasses

The scanner as described above had a horizontal bore with a diameter of approximately one-meter. This was larger than most clinical scanners and so facilitated ease of use for functional imaging as it both reduced claustrophobia and allowed good access for stimulus presentation to the subject. Because of the nature of the scanning procedure, subjects were unable to see out of the scanner and so a pair of prism glasses was used to enable subjects to see down the length of the scanner and view the projection screen.

2.4.5.2. Rear Projection & Stimulus Control

A projection screen was placed at the base of the scanner, onto which an Epson colour projector (in rear projection mode) was used to present the stimuli and instructions to the patients during the experiments. Specially written computer programmes that received TTL triggers from the scanner allowed the stimuli to be synchronised to the scanning to ensure accurate time control of the projection. The computer programmes used were all written in the department (by A. Peters and S. Francis).

2.4.5.3. Digit projection & Stimulus Control

For some studies, a rear projection device was not used. Instead a system of direct projection via a custom built LED was employed. This LED display was capable of projecting numbers to the subject. These were used in the paradigm as outlined in chapters 3 and 6. Again this projection was computer controlled (M. Humberstone) and synchronised to the scanner triggers.

2.4.5.4. Button Pressing and Control

A number of the studies (Chapters 3 & 6) required the task of button pressing as a result of the stimulation. A specific button device was made that could register the button press to ensure task completion as well as alerting when the task was performed incorrectly. It was also capable of recording the reaction time and the number of presses.

2.5. Typical fMRI Data Set

2.5.1. Slices

Individual slices are acquired rapidly during the fMRI experiment, typically 160-200 milliseconds per slice. The number of slices required to image a particular volume of brain can vary across subjects and is also a function of the slice thickness (resolution).

2.5.2. Volumes

Each set of slices of the desired brain area comprises a single volume. Each volume is typically imaged over a period of 1-2seconds depending on the number of slices per volume. Paradigms are designed with ON periods where the action or stimulation occur and OFF periods where there is time for rest and a return to baseline. Two designs are typically used, an event design which consists of a short stimulus, or a block design where the stimulus is applied over several seconds. The volume of brain has to be repeatedly imaged in order to gather the information for the whole of the time course of the paradigm.

At the beginning of a fMRI experiment, the first few volumes have a higher intensity until this effect saturates. Therefore all experiments are designed either by assigning a specific number of 'saturation volumes', which can be removed during processing, or commencing the paradigm in the OFF period.

2.5.3. Cycles

A set of volumes covering a single ON and OFF period is called a cycle and its length is termed the interstimulus interval (ISI). Ideally, we would like to identify active brain areas from a single cycle. However, in order to increase the sensitivity of experiments, stimuli must be repeated a number of times so that changes can be averaged in much the same way that neurophysiological experiments are conducted.

Typically, 20 repeats are performed. See Figure 2.16a-c.

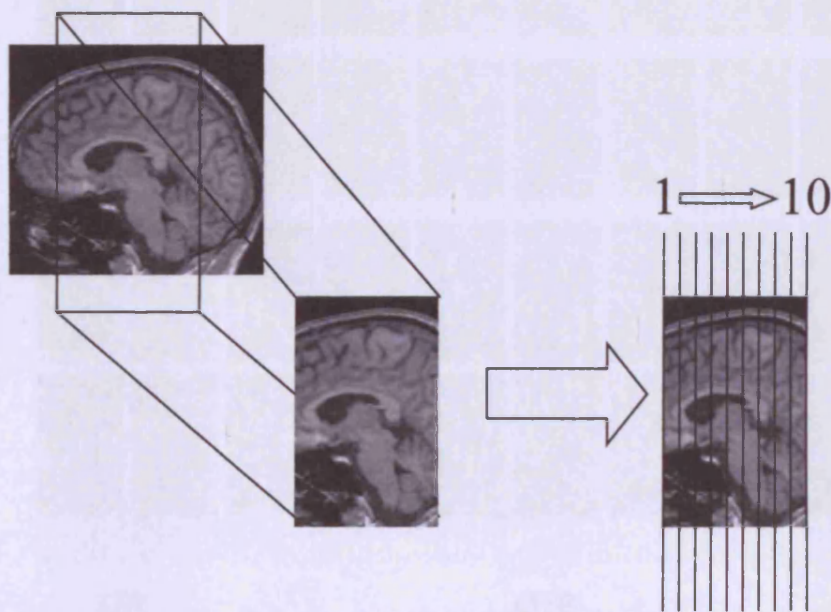


Figure 2.16a. Defining slices (1-10) of brain to be repeatedly scanned (a volume)

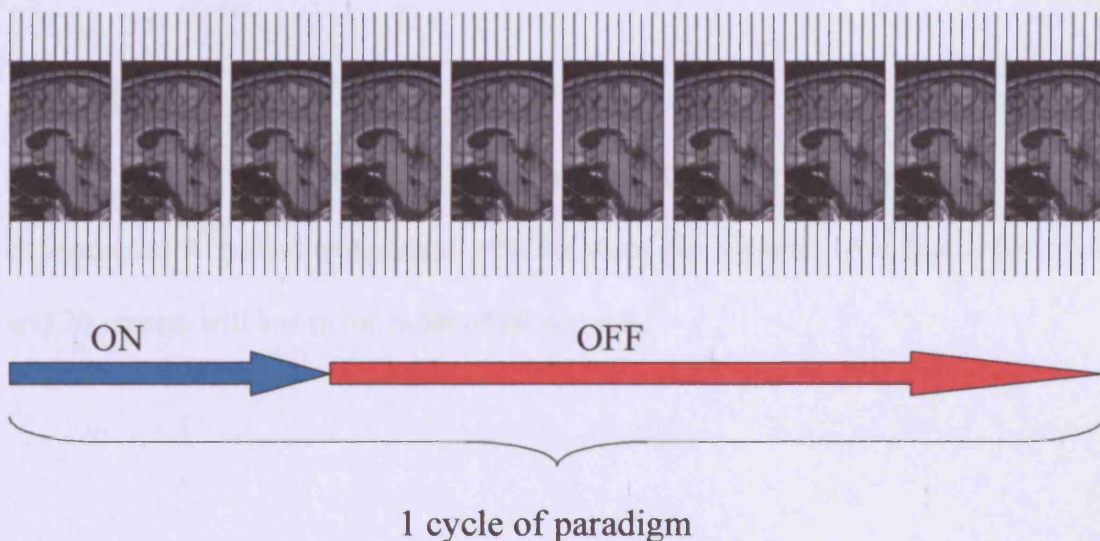


Figure 2.16b. Defining ON (3volumes) and OFF (7volumes) in a single cycle of the paradigm

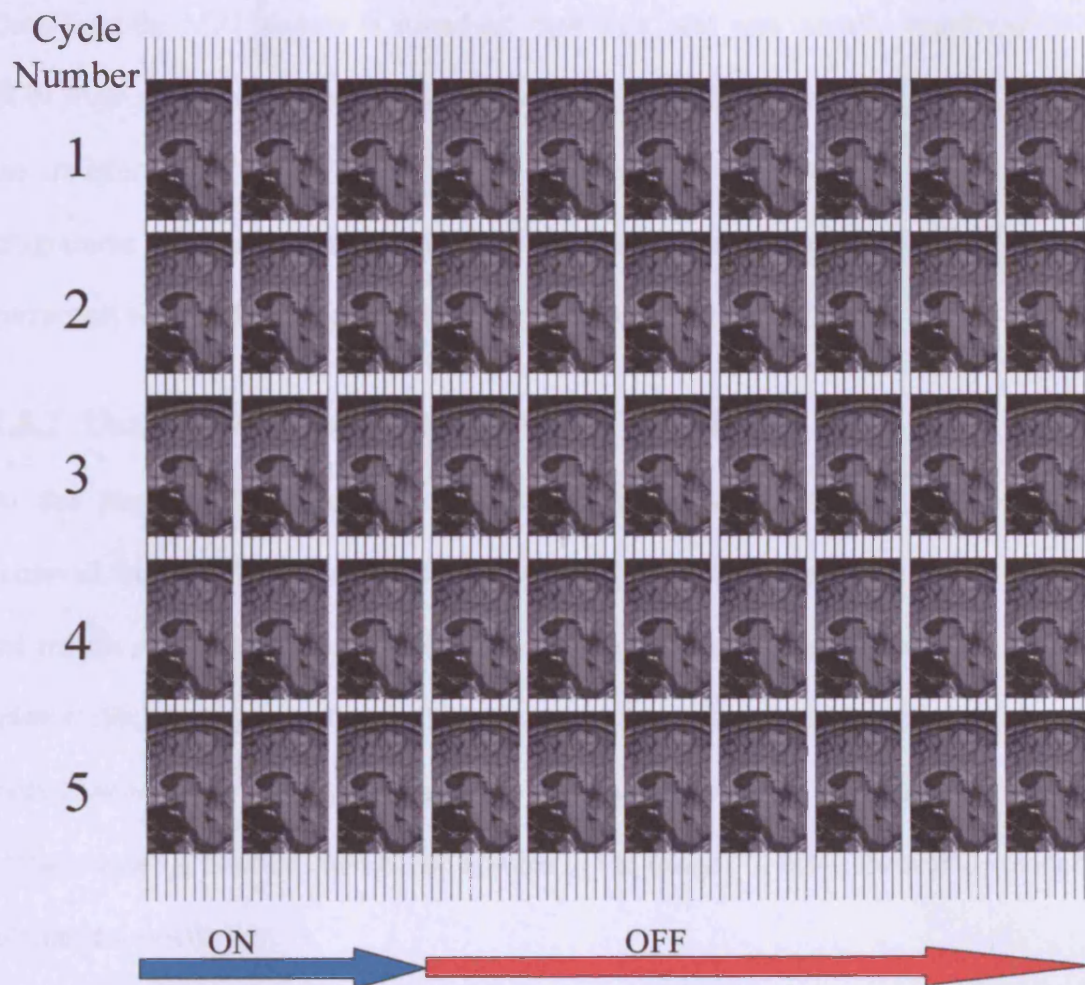


Figure 2.16c. Repeated cycles (e.g. x 5) of paradigm

2.5.4. Time

As will be clear from the description above, time becomes important in fMRI experiments. A typical experiment with 10 slices per volume, 16 volumes per cycle and 20 repeats will last in the order of 20 minutes.

2.6. Image Processing

2.6.1. Image Production

Data from the MRI scanner is stored as 'time data' and was initially transferred to a SUN solaris workstation for further processing. The first step of data processing was the transformation of the time data into image data. The data was run through a programme that performs a two dimensional Fourier transformation. At this stage, a correction for the ghost artefact (if present) was also made.

2.6.2. Data Set and Image Reduction & Rotation

At this stage, experimental pre-scan (saturation scans, see section 2.5.2.) can be removed from the beginning of the functional data series. Depending on the size of the matrix used in the scanning procedure, there is often excess matrix to the area of interest (the brain images themselves). To save time with subsequent processing, this extra matrix is removed by cropping the image down to a size that contains only the brain images. A rotation can also be applied to the images to bring them into standard anatomical orientation.

2.6.3. Global Normalisation

During the course of a fMRI experiment there may be slight changes in scanner stability, leading to a gradual global fluctuation in image intensity which could result in a reduction in the power of statistical tests. To eliminate these effects, a procedure of global normalisation is used to scale the images to the global mean of the data set.

2.6.4. Motion Correction

During the process of scanning and task performance, there are inevitable periods of movement. These are minimised by stabilisation of the head during scanning.

However, small movements of the head inevitably occur and must be corrected during post-processing. A number of different registration procedures can be used to correct for subject motion. For this thesis, motion correction was performed using the AIR algorithm (6 parameter, rigid body, MEDx, Sensor systems). This realignment algorithm corrects for motion in three dimensions: in plane and through plane movements.

2.6.5. Spatial Smoothing

Spatial smoothing of the images improves the signal to noise ratio (Oppenheim 1978), which in turn improves the ability of statistical tests to detect true activation. The smoothing applied in this thesis involved convolving the image with a three-dimensional Gaussian function, typically of width 1.5 times the voxel size. The optimal smoothing filter is one that matches the cortical activation to be identified. If the activations in the brain are highly focal then a minimal smoothing is optimal. Conversely, if brain activity is diffuse, a higher degree of smoothing should be applied

A less stringent filtering has been applied in analysis such as those in chapter 5 and 6 where highly focal activity was sought.

2.6.6. Temporal Smoothing

2.6.6.1. High Pass filtering

As well as smoothing in space, smoothing in time can also be beneficial in terms of signal to noise. Given that the BOLD effect is somewhat governed by blood flow, the rate at which genuine activation will occur in a given region of the brain is limited. It is therefore useful to filter out areas in which changes in intensity are occurring too rapidly to be real activation and therefore are artefact. By smoothing the time course

of every pixel in the image with a filter of similar shape, the signal to noise ratios can be improved. High pass filtering has been employed to remove respiratory and cardiac frequencies by convolving with a Gaussian filter of full width half maximum (FWHM) of 2.8 seconds (Friston 1995).

2.6.6.2. Low Pass filtering

As well as removing the high frequency fluctuations, it is also beneficial to remove the low frequency fluctuations. There are fluctuations in the data that are occurring far too slow to be due to real activation. Such fluctuations are caused by instabilities in the scanner hardware and again if unfiltered will affect the statistical significance of the experiment.

2.7. Statistical Analysis

2.7.1. Overview

In this thesis, a number of different methods of analysis have been utilised. The best way of comparing control and patient data in fMRI experiments hasn't yet been defined. Combinations of different forms of analysis were therefore employed and they are discussed below.

2.7.2. Defining significant activations

2.7.2.1. *t*-testing

This weighs the difference in means by the standard deviation in the predefined OFF and ON states. High *t*-scores being attributed to large differences with small standard deviations, and low *t*-scores to small differences with large deviations. One or two tailed *t*-tests maybe performed. A one tailed *t*-test is most commonly used in fMRI to test the assumption that increased neural activity leads to an increase in pixel

intensity. The above t-test requires predetermined 'OFF' and 'ON' periods. A serial t-test does not require this to be estimated. Rather a baseline OFF period is defined, as are other time points, then a t-test is performed between these and the defined off period. This yields a t-map of $N \times M$ images. Where N is the number of images per volume and M is the number of volumes.

2.7.2.2. Correlation

The correlation technique is the most commonly used statistical technique. It requires prediction of the time course, which is usually obtained by convolution of the stimulus time with the expected haemodynamic response (hrf) often crudely taken as a poisson distribution of width 6 seconds or more stringently a canonical hrf as used in SPM 99. The significance of the correlation (r) is greater the closer the agreement between the modelled hrf and raw data.

2.7.2.3 Z-Maps

Both t-test and correlation maps can be converted into probability maps, termed Z-maps. For each area identified at a given probability threshold the cluster size (k) and the percentage change (%) can be computed for further analysis.

2.7.4. Anatomical localisation of significant activations

2.7.4.1. Talairach Space

Talairach space is a set of co-ordinates that has been devised in order to map areas of activated brain to an anatomical region of the brain. The Talairach atlas (Talairach 1988) was constructed as a composite of a number of studied brains.

To define the Talairach co-ordinate system, the AC-PC line is defined using the mid-sagittal slice to locate the superior border of the anterior commissure and the inferior border of the posterior commissure. Following this a bounding box is defined, as

follows; superior border (the top of the parietal lobe), inferior border (the bottom of the temporal lobes), posterior border (the back of the occipital lobe), anterior border (the front of the frontal lobe), and laterally (the parieto-temporal lobes). The images are then rotated to align their axes with the co-ordinate system of the Talairach atlas, thus allowing improved classification of structures across subjects.

2.7.4.2. Direct anatomical assignment

For the purposes of the studies described in this thesis, this Talairach system was used in conjunction with a purely anatomical approach for activation localisation. Using anatomical atlases such as Damasio (Damasio 1995) Duvernoy (Duvernoy 1991) areas of activated areas of brain were defined on an individual basis. This has the advantage of accounting for the individual variations in anatomy rather than rotating and warping scans into Talairach space. This method is therefore more precise but extremely time consuming.

2.7.5. Region Of Interest (ROI) Analysis

Areas of the brain that are activated by a particular fMRI task, such as the motor cortex, sensory cortex etc, can be studied in greater and more accurate detail by region of interest analysis. This involves defining the whole region of interest anatomically on precise anatomical images, then using these 'maps' to sample the functional data for activity from the whole of this region during the paradigm. Adding together the activity at all the time points then creates a total activity for the region. It is best then to scale this total activity against the individual size of region and total size of brain in order to produce a normalised activity for the brain region that can be compared between groups. The principles of this analysis are similar to 'small volume corrections' available in SPM96 or 99 (Worsley 96).

2.7.6. Within-Region Spatial Analysis

In order to assess the extent of activations within a specific area, a system was devised to define a region of interest (ROI) encompassing the whole anatomical area of interest on detailed images, and then to apply this ROI to the analysis of functional data. Essentially this takes the data that is derived from the ROI analysis (section 2.7.5.) and looks at each individual map spanning an anatomically defined area in order to assess which parts of the region are activated. The data points can then be further analysed for significance using a full width half maximum (FWHM) type of analysis. See sections 4.2.5.3. and 6.2.5.

2.7.7. Distance between and orientation of significant activations

Chapters 5 and 6 study the spatial variations of active clusters between the dystonic subjects and healthy volunteers. In this assessment, the three dimensional distance (Euclidian) between the centres of activation was calculated. The centre of activation was defined as the peak area of activity within a cluster of activity (i.e. the point of most significant activation). The distance between two points on the cortex of the brain is dependent on a number of vectors, (superior-inferior, medial-lateral, anterior-posterior), therefore, to assess significance of separation between the groups, firstly a MANOVA (with vectors as dependent variables) was used, and then, if significant, a t-test employed to individually compare these different vectors. (See section 5.2.5)

2.8. Subjects

2.8.1. Normal Individuals

Subjects acting as controls in the studies were recruited under Magnetic Resonance Centre's local recruitment policy. An attempt was made to age and sex match the control subjects to the dystonic patients. A routine health-screening questionnaire

(See Appendix 1) was given to all volunteers to eliminate those with medical conditions from participation in the fMRI studies. Control subjects were also examined to rule out the presence of undiagnosed dystonia.

2.8.2. Dystonic Individuals

The nature of fMRI experiment requires subjects to lie in the MRI scanner for long periods of time. A high degree of concentration and co-operation is required in what can be a claustrophobic, noisy and hostile environment. With all these considerations in mind it, was decided that the patients with more generalised forms of dystonia would not be able to tolerate the fMRI experiments. It was also considered that patients with torticollis wouldn't be suitable for study because of potential problems with head movement during the studies. It was therefore decided that the most suitable dystonic group for study would be those with focal arm dystonia. As outlined in section 1.3 this group has been described under a number of different terminologies e.g. writer's cramp (WC), Occupational cramp (OC), Dystonic Cramp (DC) and Focal arm dystonia (FD).

For all the studies patients with focal dystonia were recruited. Patients were approached both through the local movement disorders clinics under the care of Dr Guy Sawle (Consultant Neurologist at Queen's Medical Centre) and via the local branch of the Dystonia Society (via its Chairman). A neurologist had seen all the patients and a diagnosis of focal dystonia had been made. In all patients, attempts to rule out other diagnoses had been made. The patients were further screened in an attempt to type the characteristics of their focal dystonia. A further stipulation that none of the patients should be on medication for dystonia or have received botulinum toxin therapy in the last 4 months was also made before entry into the studies.

The patients were split into groups WC, DC, OC and FD to facilitate a degree of uniformity in the studies. Other demographic details were recorded, as was the duration of their symptoms and therapies received for their dystonia. All of the dystonic subjects experienced their dystonic cramps in their dominant hands which was not clinically apparent at rest. In total, 30 patients with focal dystonia were recruited for the fMRI studies. Two of these patients participated in more than one of the studies. Two patients attended but were unable to be scanned (withdrawals due to claustrophobia and inability to visualise the stimulus screen).

2.8.3. Ethical Approval

Local ethical approval for all the studies was obtained for both the normal subjects and the dystonic patients. Under local regulations ethical approval had to be sought for control subjects and patients separately. The University ethics committee granted ethical approval for the control subjects whilst Queen's Medical Centre ethical committee granted approval for the dystonic subjects. All subjects were aware of the ethical approval at time of consent.

2.8.4. Consent

All subjects gave informed consent to participate in the studies. An information sheet outlining the nature of the study and the ethical approval was given to all study participants (see Appendix 1).

2.8.5. Safety

An information sheet (see Appendix 1) outlining the nature and potential dangers of MRI was given to all subjects prior to entry into the study. Due to the potential danger of MRI scanning, all subjects also had to satisfactorily complete a safety form (see Appendix 1) before being scanned.

Chapter Three

Cortical Activity associated with the Planning and Execution of a Simple Movement in Focal Dystonia.

3.1. Introduction

In order to study the preparatory aspects of movement, a paradigm was designed that could distinguish between the different cortical activity associated with the preparation for a movement form that is associated with execution of movement.

‘Go/no-go’ paradigms have been used extensively in neuropsychological experimentation. These paradigms consist of a two repeated stimuli, one triggering the production of a movement the other triggering no movement (with holding movement). This type of paradigm has been adapted to a number of different studies in the area of functional magnetic resonance imaging (Simson 1977, Yamanaka 2002). The ‘no-go’ section of the paradigm provokes activity associated with cognitive aspects of planning a potential movement dissociated from the action itself. The ‘go’ section provokes activity associated with both the cognitive planning as well as activity associated with the execution of the task. The paradigm selected for this experiment was known to produce robust and repeatable responses in normal subjects

form earlier studies (Humberstone 1997). From this and other studies (Ikeda 1999), there is good evidence that such a paradigm can produce two separate areas of activation in the supplementary motor cortex. These areas haven't been specifically studied in dystonia previously, although possible activation abnormalities have been alluded to in previous PET studies (Ceballos-Baumann 1995, Ceballos-Baumann 1997) where it was found that the caudal SMA was underactive whilst the rostral SMA overactive. However, other studies (Ibañez 1999, Playford 1998) have reported different findings, with the SMA being found to be overactive.

3.2. Methods

3.2.1. Patients & Controls

Six patients with dystonia and six control subjects were recruited into this study under the procedures and selection criteria outlined in section 2.8. All of the subjects were right handed. The details of the dystonic patients recruited are shown in Table 3.1.

<i>Patient Number</i>	<i>Age</i>	<i>Sex</i>	<i>Dominant Hand</i>	<i>Affected Hand</i>	<i>Dystonia Type</i>	<i>Duration of symptoms (Years)</i>
1	25	M	R	R	WC	5
2	34	M	R	R	WC	2
3	62	M	R	R	WC	15
4	21	M	R	R	DC	8
5	27	F	R	R	WC	3
6	22	M	R	R	WC	1

Table 3.1. Patient details for those participating in the study.

3.2.2. Paradigm

The paradigm (See Figure 3.1) was designed with the patient being visually presented with two numbers in pseudorandom order every 18 seconds. In response to the number '5', the subject was instructed to press a button that was placed in their right hand. In response to the number '2' the subject was instructed not to press the button. As the subjects were unaware of the order in which the numbers would be presented, but anticipated that a number would appear in a given time, the two stimuli produced two distinct states.

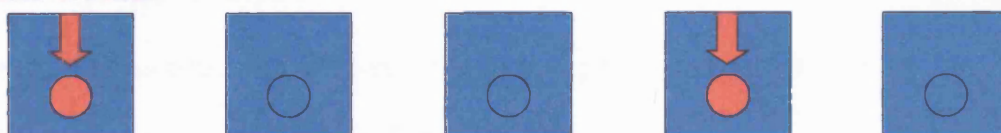
Stimulus '5' produced a period of preparation for a possible movement as well as a period of execution of the movement. Stimulus '2' produced only the period of preparation for movement.

The button pressing of the subjects was monitored throughout the experiment so that data on reaction times and compliance with task could be monitored. By comparing the results of this data with the pseudo-randomisation, 'failures to button press' when cued, inappropriate button pressing, to a 'no-go' stimulus and other 'random pressings' could be monitored.

STIMULUS



RESPONSE



Button
pressed

Button
pressing
withheld

Button
pressing
withheld

Button
pressed

Button
pressing
withheld

Figure 3.1. Paradigm Summary

3.2.3. Imaging procedure

Coronal echo-planar images with a matrix size of 128 X 128 pixels were acquired using an MBEST technique (Ordidge 1988) (in-plane resolution 2-3mm). Whole brain volumes were acquired within 12 contiguous slices, each 15 mm thick. The repeat time (TR) = 250ms and the echo time (TE) = 25ms. Each brain volume was therefore acquired in three seconds. The inter-scan interval (ISI) was 18seconds, during which six volumes were acquired. Immediately following functional data acquisition, an EPI inversion-recovery image was acquired, consisting of sixty 3mm thick slices. These had the same distortions and in-plane spatial resolution as the functional data.

3.2.4. Image Processing and Analysis

3.2.4.1. Image Processing

Image Analysis was performed on a Sun Ultra computer utilising Analyze software and the methods described in section 2.6. Images were first aligned to remove motion artefact. Images were then cropped to a matrix of 64 x 64 pixels and separated into “go” and “no-go” trials. At this stage, data series with incorrect responses and those with excessive motion or radio frequency artefacts were removed. The images were then smoothed with a Gaussian and both high and low pass temporally filtered.

3.2.4.1. Image Analysis

Initially, a serial t-test (see section 2.7.2.1.) was performed on both the ‘go’ and ‘no-go’ trials. This was done on a pixel by pixel basis at each time point using the images acquired at a time point 3-6 seconds before the stimulus delivery (the baseline period.) This generated a time series of activation maps which were then thresholded at a t-

value of 3.5 ($p=0.001$). Cluster size and percentage activation of each activated area was recorded. A region of interest analysis (section 2.7.5) was then performed on the following areas: 1. The primary motor cortex MC (pre central gyrus excluding the anterior-inferior segment), 2. The lateral premotor cortex LPMC (the anterior-inferior pre-central gyrus and the caudal part of the superior frontal gyrus), 3. The supplementary motor cortex SMA and 4. The Pre-supplementary motor cortex Pre-SMA (medial to Brodmann area 6 and caudal and rostral to the anterior commissure line respectively).

Defining these areas involved re-orientation of the anatomically precise inversion-recovery images into sagittal sections so those anatomical landmarks were visible. Using references within Talairach, Damasio and Duvernoy atlases, the cortical areas of interest were defined and mapped. These maps were then superimposed onto the functional data. Maximal percentage signal change of the whole anatomical area was then calculated and was then normalised for area size and brain volume to account for inter-subject variability. This produced a normalised percentage change value for each area under investigation. A two-tailed t-test was then applied to these normalised percentage change values to determine statistical significance between the two subject groups.

3.3. Results

3.3.1. Task Performance

3.3.1.1. Errors

No significant difference in the task performance was found between the two subject groups. The control group made a total of four errors (1.3% of presented stimuli) compared to that of six in the dystonic group (2% of presented stimuli). All errors

were an inappropriate “go” to a “no-go” stimulus. That is to say pressing the button in response to a ‘2’ stimulus. As noted previously these errors were removed from further analysis.

3.3.1.2. Stimulus reaction times

The time taken to react to the “go” stimulus tended to be quicker in the dystonic group but this did not reach statistical significance ($p = 0.36$). (Table & Figure. 3.1.)

Subject	Control	Dystonia
1	727.80	781.80
2	582.80	443.68
3	556.50	422.50
4	538.04	419.08
5	486.50	415.72
6	563.10	472.80
Mean	575.79	492.60
STDEV	81.34	143.28

Table 3.1. Average reaction times in milliseconds for all subjects with mean and standard deviation.

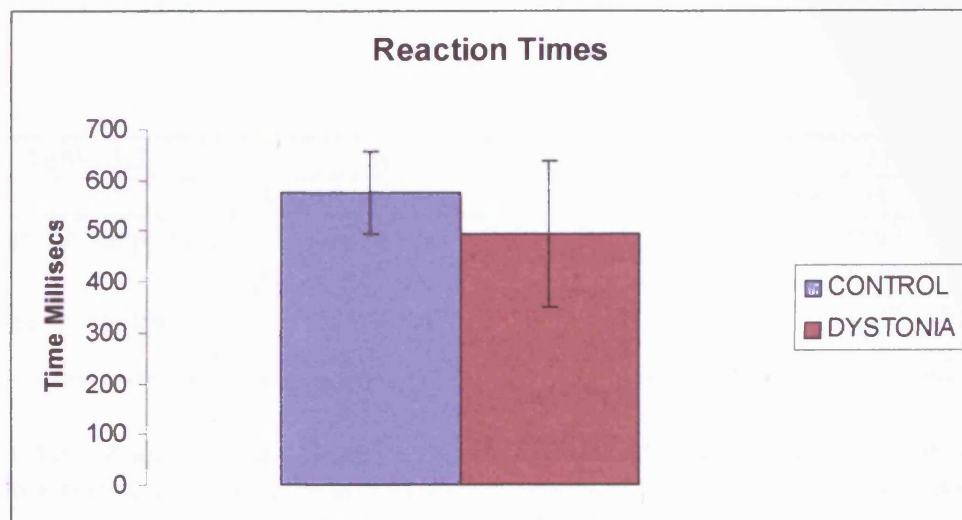


Figure 3.1. Showing the Reaction times of both groups in milliseconds.

3.3.2. Activation analysis

The main areas activated to the ‘no-go’ and ‘go’ tasks in both control subjects and patients are shown on Table 3.2. For each area, the average size of activity and the percentage BOLD signal change are shown in Table 3.3. Time courses for the activation each area studied are shown in Figure 3.2a. (the control subjects) and Figure 3.2b. (the dystonic patients). A representative sample of the activation maps seen in the SMA-proper and the Pre-SMA at 3-6 seconds after stimulus are displayed in Figure 3.3.

Table 3.2.		<i>PSMA</i>		<i>SMA</i>		<i>MC</i>		<i>LPMC</i>	
		<i>R</i>	<i>L</i>	<i>R</i>	<i>L</i>	<i>R</i>	<i>L</i>	<i>R</i>	<i>L</i>
Task									
Control	go	6	6	6	6	0	6	0	4
Subjects	no-go	6	6	0	0	0	0	0	0
Dystonic									
	go	6	6	6	6	0	6	0	5
Subjects	no-go	6	6	0	0	0	0	0	0

Table 3.2. Number of subjects in each group displaying significant activation during the go and no-go tasks.

Table 3.3.		<i>MC</i>		<i>LPMC</i>		<i>SMA</i>		<i>Pre-SMA</i>	
<i>Task</i>		%	<i>Pixels</i>	%	<i>Pixels</i>	%	<i>Pixels</i>	%	<i>Pixels</i>
Control Subjects	go	1.02	5.83	0.82	2.67	0.81	4.5	0.63	3.33
	no-go	N/S	-	N/S	-	N/S	-	0.48	3.16
Dystonic Subjects	go	0.65	3.67	1.15	4.16	N/S	3.16	0.85	5.16
	no-go	N/S	-	N/S	-	N/S	-	0.51	3.83

Table 3.3. Areas activated by the go and no-go tasks: Average percentage change and numbers of activated pixels at p=0.001 threshold. (N/S- no significant activation).

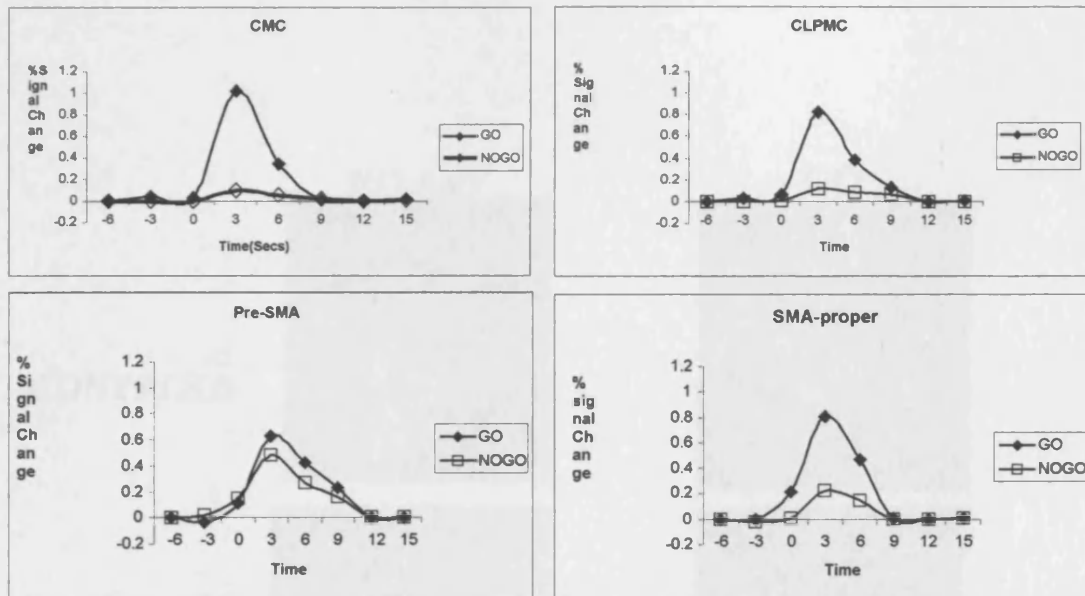


Figure 3.2a. Time course for the percentage signal change (%) in contra-MC, contra-LPMC, SMA-proper and Pre-SMA in control subjects during 'go' and 'no-go' tasks.

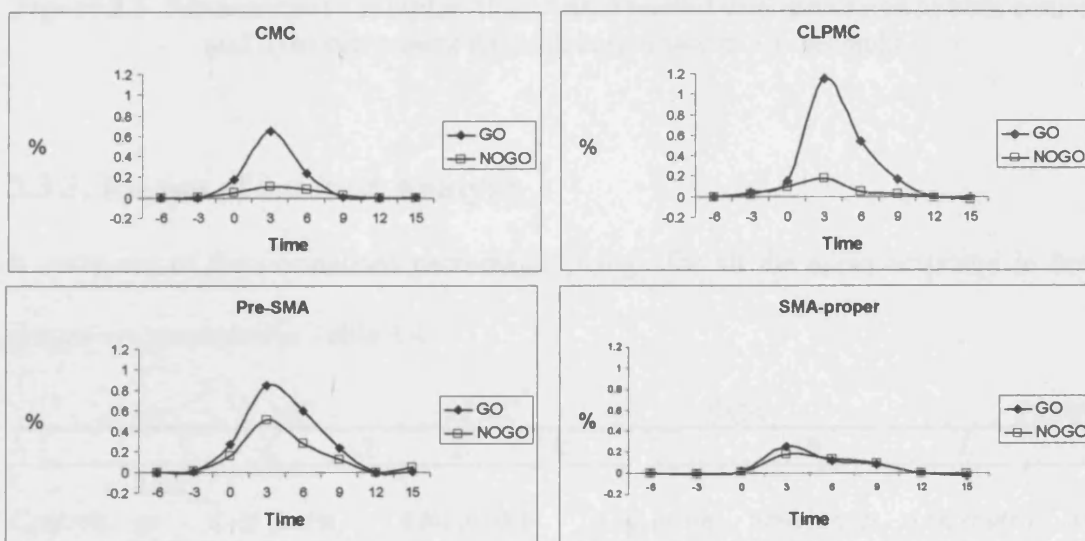


Figure 3.2b. Time course for the activations and percentage signal change (%) in contra-MC, contra-LPMC, SMA-proper and Pre-SMA in dystonics subjects during 'go' and 'no-go' tasks.

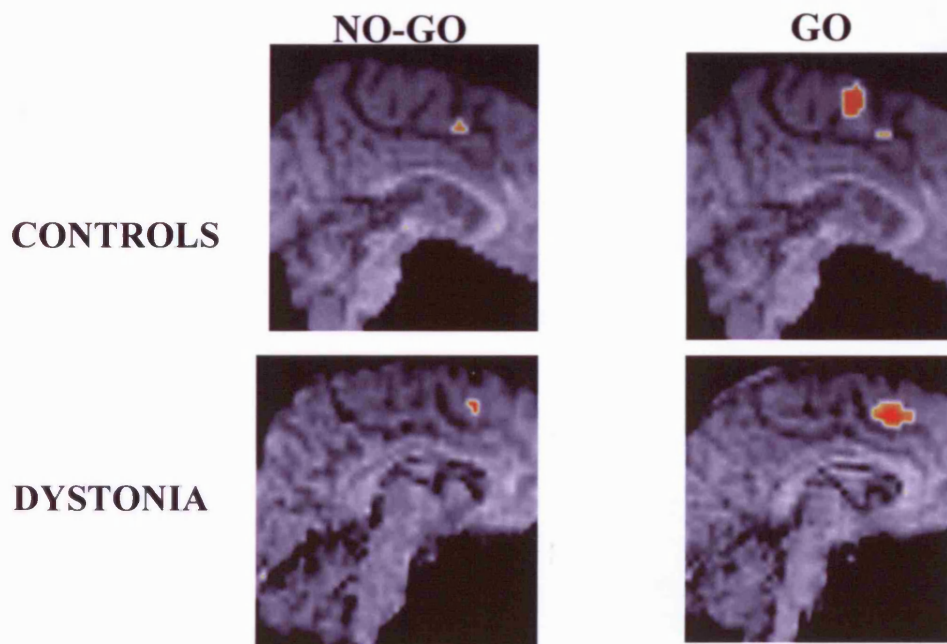


Figure 3.3. Representative samples of activated medial wall structures in both control and dystonic groups for both conditions at 3-6 seconds.

3.3.3. Region of Interest Analysis

A summary of the normalised percentage change for all the areas activated in both groups are presented in Table 3.4.

		MC		LPMC		SMA		PSMA	
		<i>L</i>	<i>R</i>	<i>L</i>	<i>R</i>	<i>L</i>	<i>R</i>	<i>L</i>	<i>R</i>
Task									
Controls	go	0.159 (0.030)	-	0.092 (0.020)	-	0.186 (0.036)	0.090 (0.032)	0.072 (0.006)	0.039 (0.007)
	nogo	-	-	-	-	-	-	0.068 (0.007)	0.040 (0.009)
Dystonics	go	0.064 (0.025)	-	0.158 (0.021)	-	0.076 (0.033)	0.022 (0.023)	0.125 (0.020)	0.0532 (0.013)
	nogo	-	-	-	-	-	-	0.064 (0.008)	0.054 (0.008)

Table 3.4. The average ‘normalised’ percentage change data across subject groups for all of the activated regions with standard deviation in brackets.

3.3.3.1. The ‘no-go’ Task

During the ‘no-go’ task there was no significant difference between the two groups in the only really activated area the Pre-SMA ($p = 0.02$ right and $p = 0.38$ left).

3.3.3.2. The ‘go’ Task

The ‘go’ task significantly activated a number of areas. Table 3.5 shows the statistically significant areas of over-activation (3.5a) and under-activation (3.5b) when compared to the control group.

Table 3.5a. Go Task		
<i>Significantly Over-active Areas</i>		
<i>Area</i>	<i>Side</i>	<i>p-values</i>
Pre-SMA	L	0.0009
LPMC	L	0.001

Table 3.5b. Go Task		
<i>Significantly Under-active Areas</i>		
<i>Area</i>	<i>Side</i>	<i>p-values</i>
SMA	R	0.0002
SMA	L	0.002
MC	L	0.0001

Table 3.5a&b. Table showing p-values for the areas of significantly over (a) and under (b) activity in the dystonic group compared to controls during the ‘go’ task.

There was reduced activity in both the ipsilateral and the contralateral SMA in the dystonic group compared to control subjects $p = 0.002$ on the right and $p = 0.0002$ on the left side.

There was a significant increase in activation in the dystonic group on the contralateral Pre-SMA but not on the ipsilateral side ($p = 0.0009$ left and $p = 0.059$ right), in comparison to controls.

The contralateral motor cortex showed significant under activity in the dystonic group ($p=0.0001$) in comparison to control subjects. The contralateral pre-motor cortex showed increased activity in the dystonic group compared to controls ($p=0.001$) during the ‘go’ task.

3.4. Discussion

3.4.1. Roles of the SMA and Pre-SMA

The concept of functional segregation of the human SMA into distinct sub-components (Pre-SMA & SMA), along the lines of that observed in the study of primates (Picard 1996, Geyer 2000), has been supported by both anatomical and imaging experiments.

Anatomically it is clear that the Pre-SMA has reciprocal connections with the prefrontal cortex (Lu 1994, Luppino 1993). The SMA has connections to the spinal cord and the motor cortex. It is also known that the SMA has a definable somatotopic organisation, whereas somatotopy in the Pre-SMA is much more poorly defined (Luppino 1995).

Imaging studies have shown that the activity of the SMA and motor cortex show a positive correlation with a task demanding increasing force or frequency, whereas, activity of the Pre-SMA shows no such correlation (Dettmers 1995, Blinkenberg 1996, Jenkins 1997, Sadato 1996). Interestingly, in one of these studies, it was also demonstrated that the basal ganglia, like the Pre-SMA, has no correlation with the force or frequency of the task. Tasks which involve pure motor imagination appear to activate the Pre-SMA in preference to the SMA (Playford 1992, Stephan 1995, Tyszka 1994).

Boecker *et al*, whilst studying increasingly complex finger movement sequences, found positive correlations with movement complexity in the Pre-SMA and the pallidum (as well as parietal and ipsilateral motor cortex.) (Boecker 1998). Another study disagreed with this notion having demonstrated that Pre-SMA activity had little correlation with sequence learning and had greater correlation with the processing or maintenance of relevant sensory information associated to the task (Sakai 2000).

Using subdural electrode placement in patients undergoing evaluation for epilepsy surgery, a 'Go/No-go' paradigm was studied which confirmed the presence of two separate areas in the medial wall (Ikeda 1999) and reached similar conclusions about the function of the Pre-SMA as Sakai *et al.*

Functional magnetic resonance imaging experiments have demonstrated activity in different areas of the medial wall premotor structures in human control subjects (Humberstone 1997). In addition, MRI 'tensor' imaging of the SMA and Pre-SMA connections has demonstrated that the motor cortex and the SMA have similar connections in the sensorimotor part of the striatum whereas the Pre-SMA sends connections to more rostral parts of the striatum including the associative areas (Lehricy 2004).

All these findings support the theory that the Pre-SMA is fundamentally different to the SMA. They suggest that the SMA has a executive motor role in movement similar to that of the motor cortex. The role of the Pre-SMA appears be more abstract, concerning planning of movements or the integration of the motor plan with sensory information into a movement execution strategy.

3.4.2. Pre-SMA and SMA-proper In Dystonic Subjects

Normal levels of activity in the Pre-SMA during the 'no-go' task and overactivity of the Pre-SMA during the 'go' task in the dystonic subjects is a curious finding. It suggests that action is required to produce abnormal activation in this area but this abnormal activation alone is not enough for the dystonia to become manifest. It may be that a certain threshold of activity has to be exceeded or some additional factor has to be involved for the excessive activity to be manifest.

From the percentage change data (Table3.3), the Pre-SMA is slightly more active during the 'go' task than the 'no-go' task in the control subjects. It therefore appears

that the activity of this area is being modulated when having to execute the planned task. It is not clear, but this modulation of activity could be the result of further monitoring of the action or due to a different ‘set’ of neurones controlling the cognitive preparation associated with action as opposed to cognitive preparation when action is withheld. Whatever the cause of this Pre-SMA activity modulation, this process appears abnormal in dystonia. Whether the over-activity of the Pre-SMA in dystonia results from lack of inhibition or excessive excitation or even if the abnormality is intrinsic to this area can only remain speculative.

SMA activity during simple movements in dystonia is also abnormal. The lack of activation of this area has been seen in other studies using PET (Tempel 1993, Ceballos-Baumann 1995, Ceballos-Baumann 1997). As this part of the SMA has motor executive functions encoding specific movement parameters, its under-activity may be explained in the same way as the under-activity of the motor cortex. (See section 3.4.3.)

3.4.3. Role of the Lateral Pre-motor cortex

Analogous to the function division of the SMA, the LPMC can similarly be divided into two separate areas. The caudal part of this structure has a number of similarities to the caudal SMA in that it has projections to the motor cortex and spinal cord, and no substantial connections to the prefrontal cortices. The rostral part of the LPMC has few connections to the motor areas and substantial prefrontal connections. This again leads to the assumption that functional segregation of the caudal areas is associated with movement execution, whilst the more rostral area is concerned with cognitive aspects of movement. This anatomical distinction of the LPMC is rarely borne out in imaging studies (Picard 2001) and indeed in the present study, the paradigm failed to elucidate two areas of activation in this area in the LPMC. This lack of distinction of

the functional components of the LPMC is probably due to the paradigm design and the spatial resolution of the imaging. Studies that have managed to separate the LPMC sub-areas have used protocols involving self-paced movements (Kollias 2001) and delayed movements (Toni 1999), as well as movements in combination with imagined movements (Gerardin 2000) (these studies often being performed at higher spatial resolution (Kollias 2001)).

The role of the LPMC (and its sub-areas) in the generation of movements appears to be gated to that of the medial premotor structures (Pre-SMA and SMA) in that it appears to sub-serve both cognitive and executable motor functions. Some differences in activations of the two premotor areas (medial and lateral premotor) have been described. In a series of experiments, Seitz *et al*, described the LPMC as being involved in ‘coding how to act in response to perceptual cues’ and ‘also what to do in relation to cue-movement associations’ so called ‘synergy encoding’ (Seitz 2000). In a review of recent literature, the role of the LPMC in transforming the intrinsic properties and spatial locations of objects into arm actions is discussed (Rizzolatti 2002). This role of the LPMC in the ‘sensory cueing’ (Freund 1996) of motor actions complements its known high level of anatomical connectivity with the parietal lobe (Wise 1997).

3.4.4. Lateral Pre-Motor Cortex In Dystonic Subjects

A number of PET studies have described activation abnormalities of the LPMC in dystonia. However, the abnormalities described have differed between studies. Over-activity in the contralateral LPMC during internally paced action has been described (Ceballos-Baumann 1995, Playford 1998) and in both LPMC during writing (Ceballos-Baumann 1995). Other studies have demonstrated under-activity of the contralateral LPMC during a writing task only (Ibañez 1999). Others describe no

differences (Tempel 1993). It has been suggested that these differences in activation probably represent subtle paradigm, patient and analysis differences between the studies (Ibañez 1999). Another possible explanation is that the activity in the LPMC in these studies represents a number of sub-areas (outlined above) and hence contamination of analysis occurs with composite rather than separate analysis.

The same criticism can be lodged against this present study and it is probable that the constraints of scanning time and comfort have meant that sacrifices with the spatial resolution of the imaging have been made. This level of resolution is probably not sufficient to these detect multiple areas. In addition to this, the paradigm failed to activate the LPMC in all subjects. This finding should therefore be viewed with caution.

Despite these reservations, it is of some interest to speculate how abnormalities in an area concerned with the organisation of the motor response to sensory cues may relate to dystonia. Whether this activity is reflective of abnormal sensory input into this area or actually represents an intrinsic abnormality of this area in integrating the information, cannot be answered at present.

3.4.5. Motor Cortex Activity in Dystonic Subjects.

The finding of under-activity of the contralateral motor cortex in dystonia is not new and has been described in a number of previous PET studies (Tempel 1993, Ceballos-Baumann 1995, Ceballos-Baumann 1997, Playford 1998). This abnormal under-activity is somewhat counter intuitive as patients with dystonia display a much greater level of motor activity. These two apparently opposing viewpoints can both be explained by the theory that there is a lack of inhibitory activity in the motor cortex. The reduction in the activity of the inhibitory neurons is sufficient to outweigh the extra activity of the excitatory neurons thereby causing a reduction in the overall

measured activity of the area. This theory is supported by evidence from TMS studies (Ridding 1995b, Chen 1997).

It is also possible that, while none of the patients reported dystonic movements with this task, there may have been subtle dystonic changes. This of course could lead to a spurious result of underactivity, as the relative change of activity (that was measured) would be less in the dystonic group

Another interesting question arising from this finding comes from the fact that this activation abnormality is detectable in a patient normally performing a task that isn't inducing their dystonia. It is possible to speculate that this asymptomatic abnormality requires a greater level of movement to pass a threshold in order to become manifest. It is also possible that specific movements or sequences of movements may activate this manifesting threshold and hence explain the task specificity seen in some of the focal dystonias.

3.5. Conclusions

Focal dystonia is associated with a number of cortical activation abnormalities in a non-writing test of the dystonic hand. We found no significant differences in cortical activation during the preparation for movement but a number of activation differences became apparent during movement. These differences were:

1. Under-activity of the contralateral motor cortex, and both the supplementary motor cortices.
2. Over-activity of the Pre-SMA and contralateral pre-motor cortex.

Chapter Four

A Study into the Cortical Activations Associated with Increasingly Difficult Actions in Patients with Focal Dystonia.

4.1. Introduction

Writer's Cramp is a task specific dystonia (Marsden 1976) in which the dystonia is induced by the act of writing. As alluded to in earlier chapters, this type of dystonia is ideally suited to be studied with fMRI, as the dystonia can be switched on and off by writing and resting.

The nature and mechanisms of the task specificity of dystonias like writer's cramp remains unclear. Why some actions are associated with dystonia and others are not is difficult to conceptualise. FMRI experiments have the ability to study a number of different tasks within the same paradigm. It was therefore hypothesised that the cortical activation abnormalities associated with the dystonic contractions could be demonstrated and contrasted within a paradigm of increasing difficulty. This

paradigm should therefore include non-dystonia provoking and dystonia provoking (writing) tasks.

From the clinical observations in dystonia, two phenomena are commonly seen. Firstly, there is overflow of muscle activity from the intended muscles to neighbouring muscles (Van der Kamp 1989). Secondly, there is co-contraction of agonist and antagonist muscles during the same movement. From this evidence alone, it is apparent that the spatial confinement of motor excitatory activity in dystonia is abnormal. Evidence from TMS may suggest that this abnormality may be detectable in the cortex (Byrnes 1998, Sommer 2002). FMRI experiments allow detailed examination of spatial activity. It was therefore hypothesised that this abnormal spatial confinement may be detectable in the motor cortex during dystonic movements but be absent during other movements.

From the results of the previous study presented in Chapter 3, there are a number of activation abnormalities in dystonia that are demonstrable in non-dystonia inducing tasks. This study also showed that there were no significant activation abnormalities associated with preparation for a simple task (non-dystonia inducing) in dystonic subjects. It was hoped that the present study would be able to show any abnormalities of activation during preparation for a movement that subjects knew, would induce dystonia.

This study was therefore planned to explore the following issues: 1) To study the spatial distribution of the activation within the motor cortex over a range of task difficulties; 2) to address the dynamic activations of brain regions during the preparation for and execution of non-dystonia inducing and dystonia inducing tasks and 3) Assess the dynamic changes in the activity of brain regions during different tasks.

4.2. Methods

4.2.1. Patients & Controls

The subjects were recruited following the guidelines outlined in section 2.8. Eight patients with writer's cramp (mean age 35.3 ± 14.4 , six males and three females) and eight control subjects (mean age 31.0 ± 8.5 , six males and three females) were scanned. All subjects except one of the dystonic patients were right handed. All of the writer's cramp patients had dystonic movements only during writing, and had dystonia evident on writing their name. None of the subjects had evidence of other neurological disease. No patients had been on any treatment for the preceding four months.

<i>Patient Number</i>	<i>Age</i>	<i>Sex</i>	<i>Dominant Hand</i>	<i>Affected Hand</i>	<i>Dystonia Type</i>	<i>Duration of symptoms (Years)</i>
1	26	F	R	R	WC	3
2	57	M	R	R&L	WC	23
3	33	M	R	R	WC	5
4	27	F	R	R	WC	3
5	37	F	L	L	WC	2
6	29	M	R	R&L	WC	7
7	62	M	R	R	WC	15
8	25	M	R	R	WC	5
9	22	M	R	R	WC	1

Table. 4.1. Showing the details of the dystonic subjects participating in study.

4.2.2. Paradigm

In order to study the cortical activation associated with dystonia-inducing task compared to a non dystonia-inducing task and to also study the preparation phases

associated to these tasks, a complex paradigm was devised. In this delayed-response paradigm, information was encoded in two messages (see Fig.4.1.& Fig. 4.2.) First, the task information was encoded. This consisted of three symbols being presented to the subject. These symbols were 'W' that encoded the writing task, '—' that encoded the line drawing (non-cramping) task and 'O' that encoded the nothing task. These 'task encoding' signals were all of the same luminance and each lasted for 900ms. Subjects held a pen in their dominant hand throughout and at the presentation of the task encoding stimulus subjects were asked to prepare for the task. 5.7 seconds later, the 'performance encoding' stimulus was shown. This 'go' stimulus was signalled by the presence of a green bar. At this signal, the relevant task was then performed (writing, line drawing or remaining still) for the duration of the 'go' stimulus (5.7sec). Subjects were all told to continue the task until the green light was extinguished. An 11.4-second rest period followed, before the next preparatory stimulus was shown. Sixty stimuli (twenty for each task) were presented in a pseudo-randomised order to the subjects. Due to the complexity of the paradigm, patients were given supervised practise of the paradigm until accurate performance was achieved. In addition, a debriefing was conducted to assess task compliance and the paper on which they drew and wrote was removed for analysis.

In effect, this complex paradigm was designed to produce six separate conditions (preparation for the three tasks, as well as execution of the three tasks) that could be analysed separately.

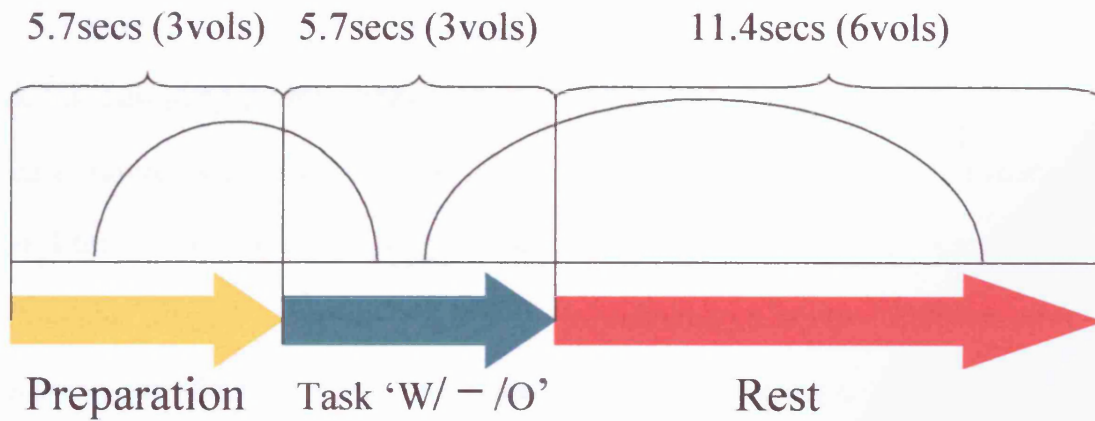
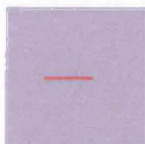
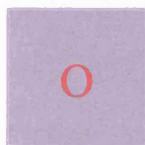


Figure 4.1. Timings of the preparation and execution of the task and the expected haemodynamic response

Task Encoding



Performance encoding

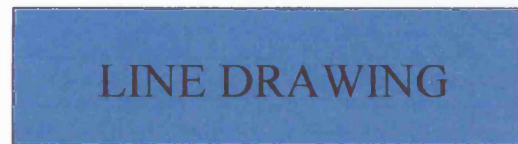


Figure 4.2. Summary of the paradigm cues and responses.

4.2.3. Imaging procedures

Each subject was scanned in a single period lasting approximately twenty-five minutes. An inflatable head restraint was used to minimise head movement. MBEST (Modulus Blipped Echo-planar Single-pulse Technique) (Ordidge 1988) was used to acquire 128 x 64 pixel echo-planar images in coronal orientation. In-plane resolution was 3mm and through-plane resolution 9mm. TE was 25ms and TR 187ms. Ten contiguous slices were scanned, comprising a partial brain volume beginning anterior to the tips of the lateral ventricles, in order to include the primary sensorimotor and pre-motor structures.

After acquisition of the functional data, anatomical images (T_2^* weighted and inversion recovery, 3mm-slice thickness) were collected using the same scanning parameters and so had equal distortions to those of the fMRI data. These images were used for anatomical registration and landmarks during data analysis.

4.2.4. Image Processing

Image analysis was performed on a Sun Ultra computer using Analyze AVW software. The images were then trimmed to 64 x 64 pixels and globally normalised. Images were co-registered using a three-dimensional AIR (Automated Image Registration) algorithm (6 parameter, rigid body). Spatial filtering was undertaken with a Gaussian filter. High and low pass temporal filters were applied. Data series with excessive motion or radiofrequency artefact were rejected at this stage. Images were separated into the three activation states of ‘nothing’, ‘line drawing’, and ‘writing’ for further analysis.

4.2.5. Image Analysis

In order to exploit the fMRI data for both its spatial and task related information, a number of different analysis methods were employed. Firstly, statistical analysis was performed in order to locate areas of interest for a more detailed region of interest analysis. Secondly, in order to test the hypothesis of spatial change in the motor cortex, a spatial analysis of the whole motor strip was performed.

4.2.5.1. Statistical Analysis

Serial t-test maps (specifically to study for activations associated with the preparatory and execution phases of the paradigm) were initially generated. These statistical maps were then overlaid onto registered anatomically precise inversion-recovery pictures as outlined in section 3.2.4.1. These maps were thresholded at $p < 0.005$ (corrected for Gaussian random fields theory). The areas significantly activated by the paradigm were studied further in ROI analysis.

4.2.5.2. Region of Interest Analysis

Analysis of the three tasks was performed on both groups. Cortical areas of interest were identified on the anatomical images as follows: - primary motor cortex MC (pre-central gyrus, excluding the antero-inferior segment); primary sensory cortex SC (the whole of the post-central gyrus); lateral premotor cortex LPMC (anterior-inferior pre-central gyrus and the caudal part of the superior frontal gyrus); supplementary motor area (SMA)-proper (medial Brodmann area 6 caudal to the anterior commissural line); Pre-SMA (medial Brodmann area 6 rostral to the anterior commissural line); rostral anterior cingulate cortex ACC and Brodmann's area 23 (the anterior part of the cingulate gyrus extending posterior to the paracentral lobule).

These regional co-ordinates were then used to extract functional data from the echo-planar images for each subject. The signal change for the each anatomical area was normalised for area size and brain volume as in section 3.2.4. A paired two-tailed *t*-test was applied to each of these normalised percentage change for the preparatory and execution phases of each activation state to identify significant differences within the subject groups. An unpaired two-tailed *t*-test was then used for direct comparison of activation changes between patient and controls.

4.2.5.3. Spatial Analysis

The motor cortex spans multiple slices. These were analysed separately and the spatial extent of half-maximum activity (along the length of the motor and sensory homunculi) was determined as a measure of the distribution of activation. The patient and control results were compared using an unpaired *t*-test.

4.3. Results

4.3.2. Task Completion

The debriefing interviews with the subjects showed that the paradigm was easy to follow and the tasks were completed with very few errors. The dystonic patients reported dystonic cramps during the writing task but this was not universal, and some patients found that the dystonic cramping during writing was less severe when they were lying in the scanner than when sitting at the desk. None of the dystonic patients complained of symptomatic cramps in the line drawing task. Task completion and accuracy was further assessed by examination of the paper on which the lines and signatures were written.

4.3.3. Statistical Analysis

4.3.3.1. Activation during Preparation

During this task there was significant activation in multiple cortical areas. The most consistently activated areas were Pre-SMA, SC, SMA, LPMC, ACC and Broca's area. The ipsilateral cerebellum was also activated in a few cases. Figure 4.3.

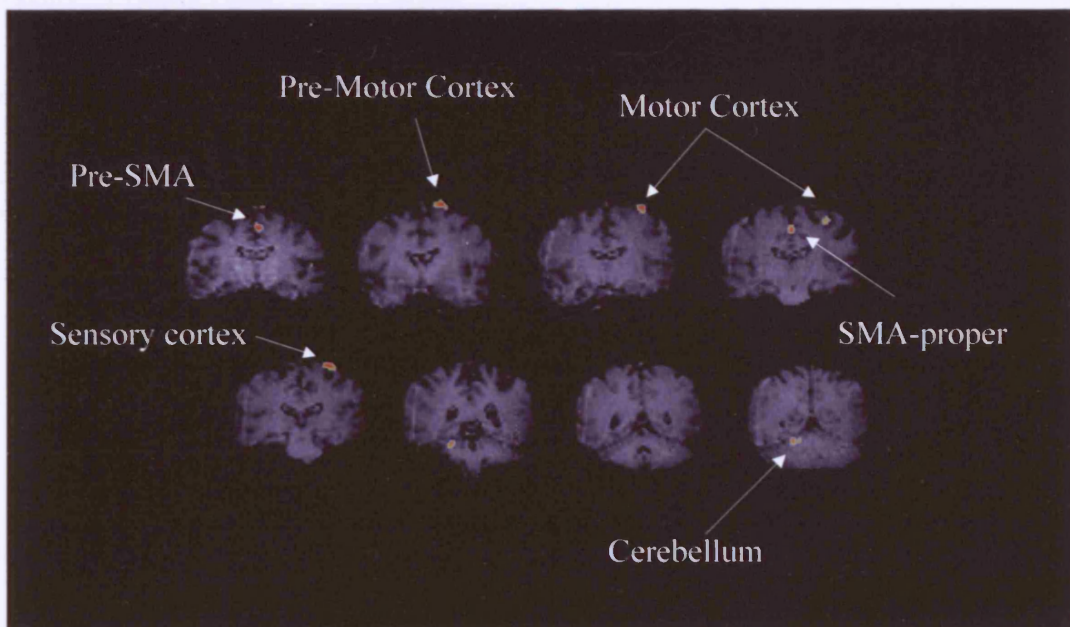


Figure 4.3. A representative picture of the activation achieved during the preparative tasks

4.3.3.2. Activation during Execution

During the execution phase, consistent areas of activation were found in the MC, SC, LPMC, SMA-proper, Pre-SMA and anterior cingulate cortex (ACC) in both the line drawing and the name writing tasks. Figure 4.4. a & b. show an example of the activation found during the writing tasks in control and dystonic subjects. Broca's area was also activated by the task but less consistently. The ipsilateral cerebellum was also evident in some subjects but distortions of this area due to scanning

parameters meant that this was inconsistent and unreliable; therefore further analysis of this area was not undertaken.

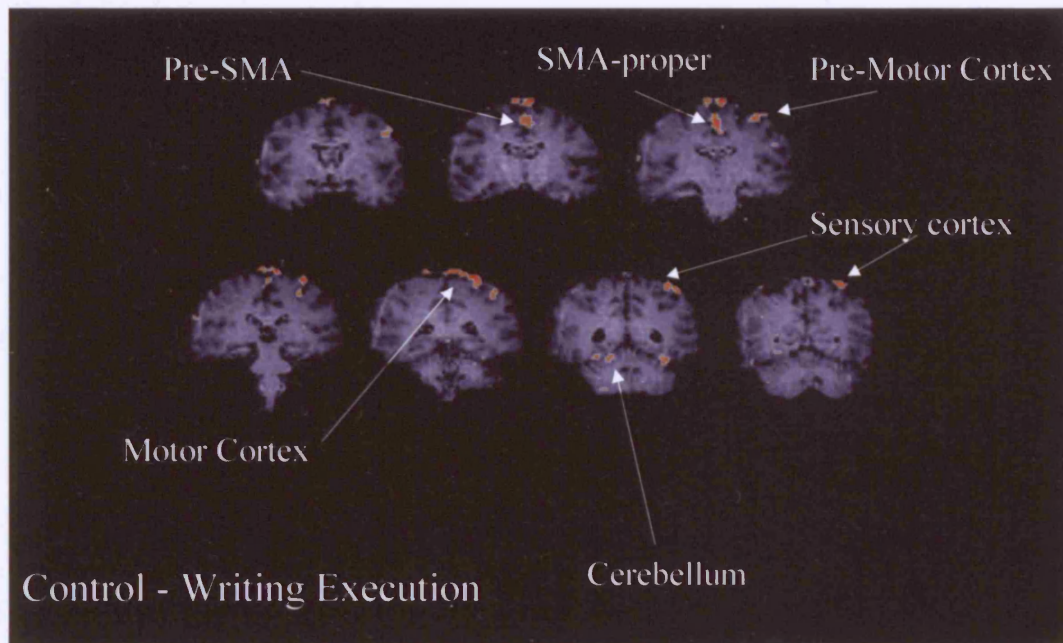


Figure 4.4a. Significantly activated areas during execution of the writing task in control subjects

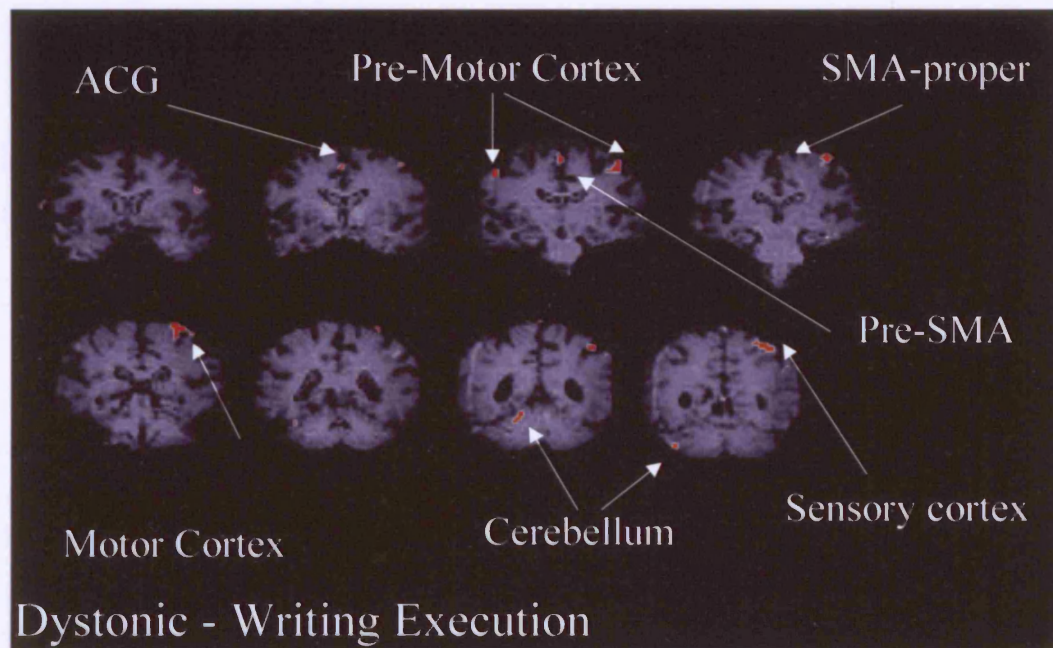


Figure 4.4b. Significantly activated areas during execution of the writing task in dystonic subjects

4.3.3. Region of Interest Analysis

4.3.3.1. Activation during preparation period

4.3.3.1.1. Control subjects

In comparison to baseline, when preparing for the ‘nothing’ task the control subjects activated their contralateral sensory cortex and SMA-proper. When preparing to draw a line, there was widespread bilateral activity seen in the sensory, motor and premotor cortices and contralateral activation of Pre-SMA and SMA-proper. When preparing to write their name, control subjects activated their primary motor cortex contralaterally and all other sampled areas bilaterally.

In comparison to the preparation for the ‘nothing’ task, when control subjects prepared to draw a line or write their name, there was significant additional activation of contralateral primary sensory cortex ($p = 0.008$ prior to line drawing and $p = 0.005$ prior to writing) and primary motor cortex ($p = 0.035$ prior to line drawing and $p = 0.006$ prior to name writing). Summaries of these results are displayed in tables 4.2 a & b.

4.3.3.1.2. Patients with Dystonia

In comparison to baseline, the dystonia patients showed no activation when preparing for the ‘nothing’ task. They activated contralateral motor, sensory and Pre-SMA cortices as well as the premotor cortex bilaterally during preparation for line drawing. When preparing to write, they activated motor cortex and anterior cingulate contralaterally and all other sampled areas bilaterally.

In comparison to the preparation for the ‘nothing’ task, when preparing to draw a line or write their name, there was no significant additional activation of primary motor or sensory cortex, but there was additional contralateral activity in the Pre-SMA (line drawing $p = 0.02$, name writing $p = 0.0013$) and ipsilateral premotor cortex ($p = 0.01$ prior to line drawing, $p = 0.0009$ prior to name writing). Prior to name writing, in addition to the structures activated in line drawing task, the contralateral anterior cingulate activity was increased ($p = 0.016$) as was ipsilateral Pre-SMA ($p = 0.003$). A summary of these results are displayed in tables 4.2a&b

<i>Line Drawing</i>		
<i>Preparation</i>	<i>Controls</i>	<i>Dystonics</i>
Area of Brain		
C- SC	0.008	n/s
C-MC	0.035	n/s
C-LPMC	n/s	0.01
C-Pre-SMA	n/s	0.02

Table 4.2a. Summary of the significantly activated areas during the preparatory tasks Preparation for line drawing versus preparation for nothing task: p values for different areas. (n/s = not significant (C) contralateral (I) ipsilateral)

<i>Writing Preparation</i>	<i>Controls</i>	<i>Dystonics</i>
Area of Brain		
C-SC	0.005	n/s
C-MC	0.006	n/s
C-PMC	n/s	0.0009
I-PMC	n/s	0.045
C-ACC	n/s	0.016
C-Pre-SMA	n/s	0.0013
I-Pre-SMA	n/s	0.003

Table 4.2b. Summary of the significantly activated areas during the preparatory tasks Preparation for writing versus preparation for nothing task: p values for different areas. (n/s = not significant (C) contralateral (I) ipsilateral)

4.3.3.1.3. Patients Vs controls

A direct comparison of patient and control data revealed a significant increase in Pre-SMA signal during preparation for both the line drawing ($p = 0.029$) and name writing ($p = 0.034$) tasks. In addition, there was an increase in ipsilateral premotor cortex signal in preparing for the writing task ($p = 0.04$). There was also underactivity of the contralateral motor cortex ($p=0.01$) during this preparatory phase. This is summarised in table 4.3.

<i>Area Of Brain</i>	<i>Preparation:</i>	<i>Preparation:</i>
	<i>Line Drawing</i>	<i>Writing</i>
Overactive	<i>p-value</i>	<i>p-value</i>
C- Pre-SMA	0.029	0.034
I-LPMC	n/s	0.04
Underactive		
C-MC	0.031	0.011

Table 4.3. Summary of the significant activation in the preparatory tasks: comparison of patients and controls. (n/s) refers to non-significant changes, (C) to contralateral and (I) to ipsilateral.

4.3.3.2. Activation during main (execution) task

4.3.3.2.1. Control subjects

In comparison to baseline, when engaged in the nothing task, the control subjects activated contralateral anterior cingulate, Pre-SMA and SMA proper. When drawing a line, they activated primary motor cortex and SMA proper bilaterally, as well as premotor cortex and sensory cortex contralaterally. During name writing they activated contralateral sensory, motor, and premotor cortex, as well as bilateral activation of the anterior cingulate cortex, Pre-SMA and SMA proper.

When the line drawing task was compared with the nothing task, there were no additional significant areas of activation. On comparison of the writing task with the

nothing task there was significant additional activation of contralateral primary sensory ($p = 0.03$) and motor ($p = 0.002$) cortices. Summaries of these results are displayed in table 4.4.a&b.

4.3.3.2.2. Patients

In comparison to baseline, when the dystonia patients were engaged in the nothing task, they activated sensory cortex bilaterally as well as ipsilateral premotor cortex and contralateral pre-SMA. However, both drawing a line or writing their name activated primary motor cortex contralaterally and all other sampled areas bilaterally.

When the dystonic patients' line drawing task was compared to the nothing task, they showed additional activation of contralateral motor cortex ($p = 0.02$). On comparison of the writing task with the nothing task, significant activation of the contralateral motor cortex ($p=0.004$) and contralateral sensory cortex ($p=0.001$) was found. During both line drawing and writing tasks, there was a significant increase in SMA proper activity ($p=0.001$ for line drawing and $p=0.02$ for name writing) and in anterior cingulate ($p=0.011$ for line drawing, 0.008 for name writing). Summaries of these results are displayed in table 4.4.a&b.

<i>Line Drawing</i>	<i>Controls</i>	<i>Dystonia</i>
Area of Brain		
C-MC	n/s	0.02
C-SMA	n/s	0.001
C- ACC	n/s	0.011

Table 4.4a. Summary of the significantly activated areas during the execution tasks
Execution of line drawing versus execution of nothing task: p values for different areas. (n/s = not significant (C) contralateral (I) ipsilateral)

<i>Writing</i>	<i>Controls</i>	<i>Dystonia</i>
Area Of Brain		
C-SC	0.03	0.001
C-MC	0.002	0.004
C-SMA	n/s	0.02
C-ACC	n/s	0.008

Table 4.4b. Summary of the significantly activated areas during the execution tasks
Execution of writing versus execution of nothing task: p values for different areas.
(n/s = not significant (C) contralateral (I) ipsilateral)

4.3.3.2.3. Patients Vs controls

Direct comparison between patient and control data revealed no significant differences in activity during the line drawing task. However, during the writing task the patient group showed significant over-activity of contralateral sensory cortex and under-activity of contralateral SMA proper and the motor cortex.

		<i>Execution: Line</i>	<i>Execution:</i>
<i>Area Of Brain</i>		<i>drawing</i>	<i>Writing</i>
Overactive		<i>p-value</i>	<i>p-value</i>
	C-SC	n/s	0.038
Underactive			
	C- SMA	n/s	0.032
	C-MC	n/s	0.021

Table 4.5. Summary of the significant activation in the writing execution task, comparisons of patients and controls. (C) Refers to contralateral.

4.3.3.3. Changes in spatial extent of Motor Cortical activation

The time taken to reach maximal activation in the motor cortex did not differ significantly between groups ($p=0.83$), the average peak occurring in the eighth volume in each case. During the line drawing and name writing tasks, activation of the motor cortex in the dystonic patients covered a greater area than in the controls (p

= 0.12 for line drawing and $p = 0.03$ for name writing). Figures 4.4 & 4.5 display the average activity across the motor cortex during the whole of the execution phases of line drawing (Figure 4.4.) and writing (Figure 4.5.) Despite this increased spatial extent of activation, the peak level of activity within the active motor cortex during name writing was significantly reduced in comparison to the control group ($p = 0.04$). Figure 4.6 displays the evolution of the spatial activity within the motor cortex at different time points of the writing paradigm.

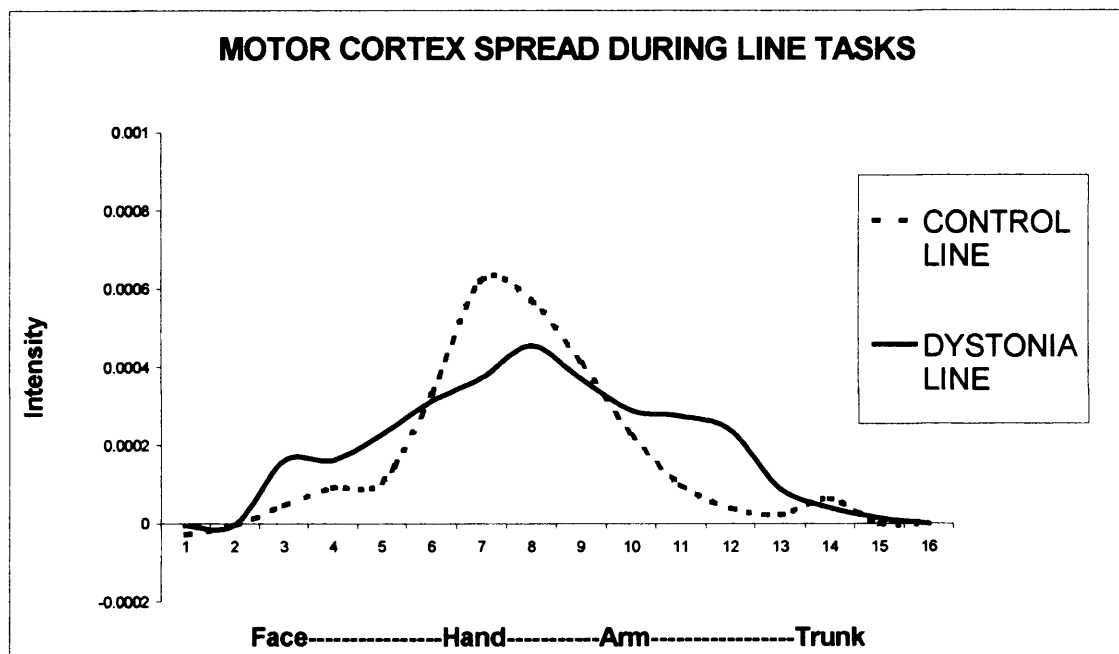


Figure 4.4. Spatial activation within the Motor Cortex of subjects during the line drawing task.

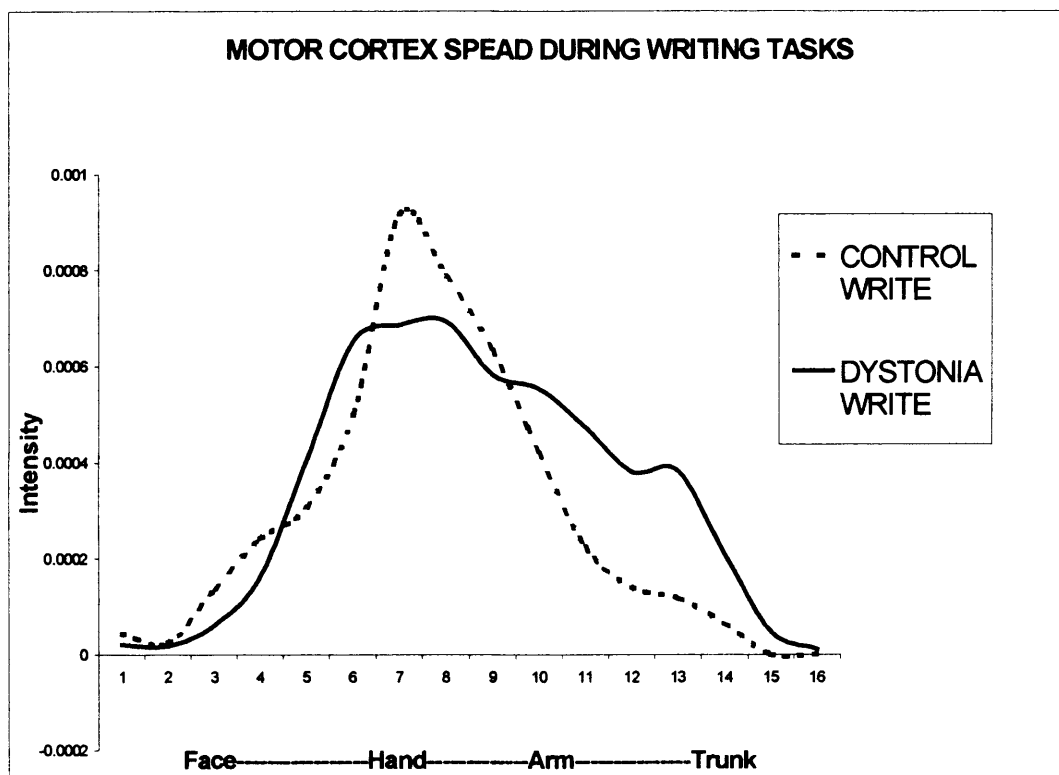


Figure 4.5. Spatial activation within the Motor Cortex of subjects during the writing task.

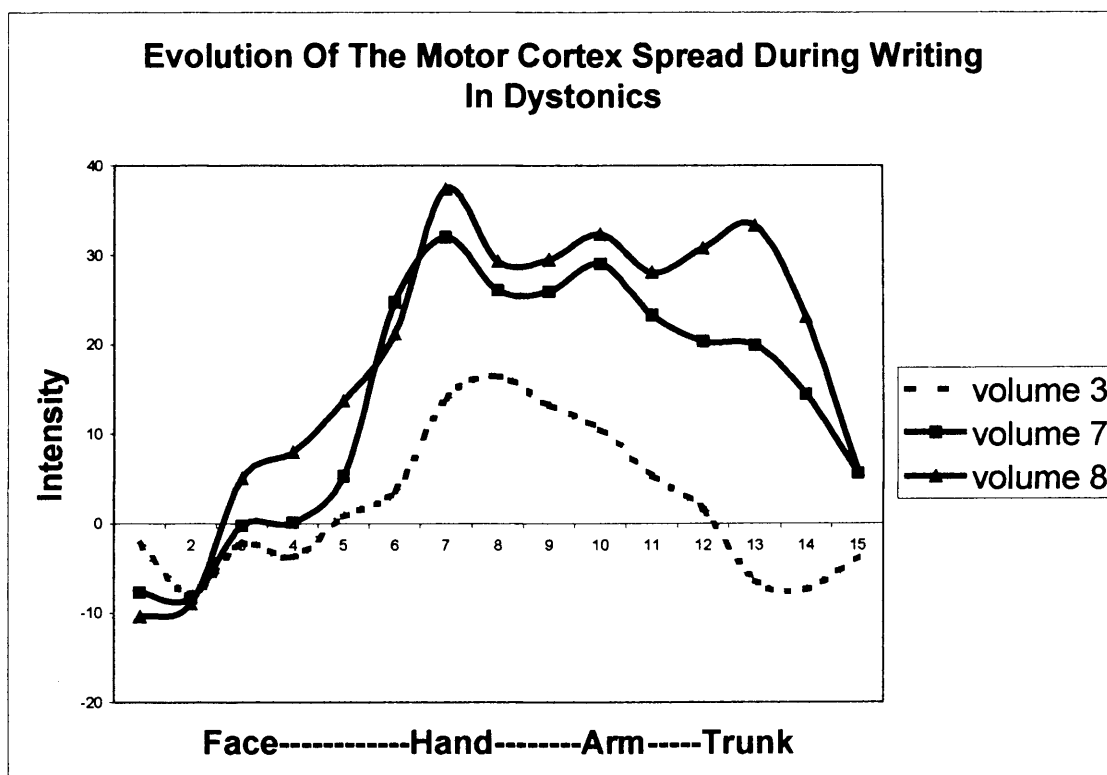


Figure 4.6. Evolution of spatial activations throughout the writing paradigm.

4.4. Discussion

Although the symptoms of writer's cramp occur only during writing, this study demonstrated abnormal cortical activation during the preparation for a range of tasks, including line drawing, name writing and remaining still.

4.5.1. Abnormalities during preparation

The main findings during the preparation phase were:

- 1) The control subjects (but not the patients) activated sensory cortex and SMA proper when preparing to remain still.
- 2) When preparing to draw a line or write, the patients showed less activation of the sensory and motor cortices than the control subjects, but greater activation of Pre-SMA, Ipsilateral premotor cortex and contralateral anterior cingulate.

In a previous PET study of patients with generalised dystonia performing paced joystick movements in freely chosen directions, Ceballos-Baumann and colleagues reported significant over-activity in lateral premotor cortex, rostral (Pre-) SMA, anterior cingulate area 32, and significant under-activity was found in caudal SMA (proper), and posterior cingulate (Ceballos-Baumann 1995). These data were collected in 90-second epochs combining the selection of movement direction and subsequent movement execution. In a subsequent study, the same authors scanned subjects with writer's cramp while writing the word 'dog' every 4 seconds. This paradigm was chosen to minimise 'thinking time' and to maximise activity in the motor output circuits. This study showed impaired activation of the caudal SMA and anterior cingulate, and over-activity of the ipsilateral premotor cortex in comparison to control subjects (Ceballos-Baumann 1997). The findings in this study complement and expand these studies, showing that the Pre-SMA and ipsilateral premotor cortex changes occur during preparation for movement, and that sensory cortex activity continues at

an enhanced level throughout execution during which the SMA-proper fails to activate fully.

In a PET study involving sentence writing, Ibañez and colleagues reported under-activity in contralateral premotor cortex (Ibañez 1999). We were not able to confirm this finding in our study, as the contralateral premotor cortex was similarly active in both subject groups during the preparatory and execution phases of both line drawing and name writing. Our patients were asked to write in 5.7-second epochs, whereas the patients in the Ibañez study wrote the phrase ‘the book is on the desk’ repetitively for over one minute. It is possible, therefore, that contralateral premotor under-activity occurs during the execution of longer writing tasks.

We found over-activity of the ipsilateral premotor cortex during preparation for both line drawing and writing tasks. Ipsilateral premotor activation has also been identified in PET activation studies involving writing (Ceballos-Baumann 1997). This may be a consequence of abnormal connectivity between the premotor cortices or relate to their specialist functions. For example, it has been demonstrated that there is a degree of left pre-motor dominance for ‘rapid response’ movements to visual cues (Schulter 1998).

The roles of the anterior cingulate cortex are believed to include attention, sensory modulation, pain perception and preparatory functions in motor control (Allman 2001). Primate data suggests functional regional specialisation, and although functionally separate areas have yet to be identified in humans, there is acceptance that areas in the cingulate gyrus that have roles in both affect and cognition (Devinsky 1995). Primate studies suggest a role in preparation and execution of both simple and complex movements (Talairach 1973). Previous studies in dystonia have shown the anterior cingulate to be over-active during freely selected joystick movements

(Ceballos-Baumann 1995), and under-active during stereotyped word writing in patients with writer's cramp (Ceballos-Baumann 1997). The apparent conflicting nature of these reported results may simply reflect the complex functional organisation of the ACC motor areas and sampling problems in the studies. The anterior cingulate was over-active in this study during the preparation for name writing in dystonic subjects.

As demonstrated and discussed in the previous chapter, the SMA is divided histologically and functionally into Pre-SMA and SMA-proper (Humberstone 1997, Picard 1996, Dieber 1991, Vorobiev 2000). The Pre-SMA is rostral and some of its output is mainly directed towards the SMA proper (Kandel 2000) which in turn projects to the primary motor cortex and to the corticospinal tracts. Our dystonic patients over-activated their Pre-SMA bilaterally during preparation for both motor tasks. Increased Pre-SMA activity has been demonstrated previously in dystonia (Ceballos-Baumann 1995). In a repetitive writing task Ceballos-Baumann and colleagues found Pre-SMA over-activity only when a less rigorous statistical threshold was set. This over-activity was no longer evident when the patients were treated with botulinum toxin, raising the possibility that this over-activity was a secondary phenomenon. Our data suggest this is not the case, as Pre-SMA over-activity occurred prior to either line drawing or name writing, indicating an abnormality in motor planning, rather than a response to involuntary movement.

The under-activity of the motor cortex in dystonia has been discussed previously in section 3.3.3.2. The present study adds to the findings in chapter three by demonstrating that this area is underactive during the preparation for action (as well as during execution). It is not clear why the motor cortex is active during the preparatory phase before a movement. This is unlikely to be contamination of the paradigm

(although this cannot fully be ruled out) and may relate to motor imagery. It has previously been demonstrated that motor imagery can activate the motor cortex (Lotze 1999, Stippich 2002, Ehrsson 2003), although this may only be a transient phenomenon (Dechent 2004). The under activity of the area in the dystonic subjects probably represents a failure of this preparatory/planning function of the motor cortex which clearly continues during the execution of the task. Whether this failure of ignition of the motor cortex is a primary problem or is driven by the overactive 'planning' areas in dystonia cannot be answered by this study.

4.5.2. Abnormalities during execution

The main findings during execution were:

- 1) There were no significant differences between patients and controls during either the nothing or the line drawing tasks.
- 2) Patients showed over-activity in contralateral sensory cortex and under-activity of the SMA-proper during execution of the writing task.
- 3) In contralateral primary motor cortex there is underactivity despite patients activating a wider cortical area than the controls. The peak activation in patients was of a lower magnitude than in control subjects.

The SMA-proper has outputs to the motor cortex and to the corticospinal tracts. It encodes a variety of movement parameters including frequency and force (Dettmers 1995) and is involved in movement sequence control (Boecker 1998, Lee 2003), especially in bimanual (Immisch 2001) and externally cued movements (Thickbroom 2000). It may also have a role in sensation perception (Naito 1999). Impaired activation has been shown in dystonia patients in previous PET studies (Tempel 1993, Ceballos-Baumann 1995, Ceballos-Baumann 1997). In our patients, the SMA-proper was slow to activate during all preparation tasks, and during line drawing, although

these did not reach statistical significance. However, when patients were writing their name, there was a statistically significant disengagement of contralateral SMA-proper activity. This finding mirrors previous studies that showed under-activity of the SMA during vibrotactile stimulation that induced contraction in the hand of patients with writer's cramp (Temple 1993). Therefore, it may be that the failure of the SMA to increase its activity further during the demanding task of writing has an integral part to play in dystonia generation. However, it is also plausible that the presence of the dystonic contraction causes the disengagement in SMA activity.

Sensory abnormalities are recognised in dystonia (Hallett 1995). Sensory tricks are common, suggesting a significant role at least in the attenuation of dystonic postures. Recent studies have demonstrated altered homuncular organisation of the sensory cortex (Bara-Jimenez 1998, Elbert 1998). Previous PET studies in dystonia have reported either decreased (Tempel 1990, Tempel 1993, Ceballos-Baumann 1995), or increased (Ceballos-Baumann 1997) activation of primary sensory cortex during motor activity. In the present study, control subjects activated their contralateral sensory cortex during name writing but not line drawing. This could either be related to enhanced sensory feedback during name writing, or it may reflect a role in motor output, since both the primary and secondary sensory cortices have projections to the spinal motor pool (Kandel 2000).

Compared to our control subjects, our dystonic patients' contralateral sensory cortex had enhanced activation during name writing. Again, the possibilities are either that this is a reflection of sensory feedback secondary to the dystonic movements, or else it reflects a contribution of the sensory cortex to motor output. Although it failed to reach statistical significance ($p = 0.07$), the dystonic patients' contralateral sensory cortex was modestly over-active during line drawing, even though no dystonic

movements were evident. This supports an abnormality of sensory processing in dystonia, beyond simple perception of the dystonic movements.

The primary motor cortex has a role in the control of complex finger movements (Gerloff 1998); this may be achieved by selective filtration of sensory information and by co-ordinating activity in descending systems (Canedo 1997). The finding of abnormal primary motor cortex activation in dystonia is not new, but the finding in this study of a broader area of activation at a lower peak level is novel. Explanations as to why this counter-intuitive finding underactivity may arise are discussed in section 3.4.5. Additionally, and specifically with this paradigm, contamination of motor activity due to the repeating nature of the paradigm, and the possibility of ongoing dystonia affecting the measured change in the dystonic motor cortex, are both possible confounding factors

Previous PET activation studies have reported under-activity in the motor cortex (Tempel 1990, Tempel 1993, Ceballos-Baumann 1995, Ceballos-Baumann 1997, Ibañez 1999, Playford 1998) and have suggested, since this is unaffected by treatment with botulinum toxin, that this could be a fundamental abnormality in dystonia. We also found a reduction in the peak level of motor cortex activation, but this was coupled with a broader spread of activity across the motor strip. This widespread activation of motor cortex occurs mainly during name writing, but is also seen to a lesser extent during line drawing. We hypothesise that this broadening of activation underpins the overflow of muscle contraction in dystonia. This overflow of activity may be a result of an alteration in cortical input or abnormal inhibition within the motor cortex itself.

In 1996, Mink proposed the centre-surround theory of basal ganglia program generation (Mink 1996). It proposes that the inhibition of motor program generators

(MPG) involved in the specific movement are relaxed as inhibition of competing MPG is increased. This allows a specific motor program to run without competing influences. It can be postulated that if there is a 'blurring' of the normally sharply demarcated boundary between the central core of activity and surrounding inhibition, there will be a resultant overflow of activity within the motor cortex into the surrounding areas causing normally inhibited muscles to become activated. Further studies in dystonia using transcranial magnetic stimulation suggest an inability to focus activity in the motor cortex. Confirming a lack of inhibition and an excess of facilitation in the area surrounding activation within the motor cortex (Sommer 2002, Chen 1997, Garcia de Casasola 1998). The findings of this study are compatible with this failure of centre-surround inhibition, leading to a lowered peak activity with spread into adjacent cortex causing unwanted muscle activation this will be elaborated on further in section 7.1.2.1.

It is still not clear, however, whether this is as a result of an abnormal program pattern generated by the basal ganglia, or a local phenomenon due to motor cortical pathology, or both. However, given the broader picture of activation abnormalities in the cortex and that fact that motor cortical damage doesn't cause dystonia, the arguments for abnormal basal ganglia action have more credence.

4.5. Conclusions

In relation to the original hypotheses, this study demonstrated:

- 1) Preparation to write was only associated with excessive LPMC activity in addition to preparation for line drawing.
- 2) Dynamic changes in the SC, MC and SMA correlated best to the production of dystonia.

3) Overflow of motor cortical activity was demonstrated in patients with dystonia.

Chapter Five

An fMRI Study of Cortical Sensory Activation in Focal Dystonia

5.1. Introduction

Despite dystonia being manifestly a motor disorder, as mentioned in chapter one there are also a number of sensory abnormalities. The consideration that the sensory system may be of paramount importance in focal dystonia (Hallett 1995) has come about through a number of observations. From a clinical standpoint, three pieces of information are key. Firstly, focal dystonia may develop following sensory injury. This injury may be within the central (Ghika 1998, Weiner 2001) or peripheral nervous systems (Jankovic 2001). Secondly, the presence of sensory tricks (geste antagoniste) implies at least some form of sensory induced modulation of dystonic contractions, perhaps mediated by the parietal cortex (Naumann 2000). Thirdly, attempts at treatment of dystonia with anaesthetic block (Kaji 1995b) have met with some success. Aside from clinical evidence, there has also been a growing amount of experimental evidence for the presence of sensory abnormalities in the pathophysiology of dystonia. Positron emission tomography (PET) has demonstrated a variety of abnormalities within the primary sensory cortex (SI) and parietal cortex depending on the manner of stimulation or the task involved. In particular, it has

been demonstrated that the contralateral primary and secondary sensory cortices are overactive during writing activity (Ceballos-Baumann 1997), while conversely, during tonic vibration, the sensorimotor cortex was found to be under active in comparison to controls (Tempel 1990). The sensory system of dystonic patients has also recently been shown by electrophysiological methods (Bara-Jimenez 1998) and magnetic source imaging (Elbert 1998), to possess an abnormality in the cortical representation of individual digits. Specifically, there appears to be a fusion of digit representations and a disruption of the normal homuncular arrangement in the somatosensory cortex of the affected hand. Most recently, further evidence for a dysfunction of the sensory system in dystonia has been described using somatosensory discrimination tasks (Tinazzi 1999, Bara-Jimenez 2000a&b). These observations corroborate earlier work on monkeys showing that repetitive movements can induce changes in sensory cortex, results that have led to the primate model for dystonia (Byl 1996).

So far, studies have mainly concentrated on the primary sensory cortex (SI) as a whole only. However, within the primary sensory cortex itself there are a number of sub-regions involved in the integration and distribution of sensory information.

To explore activation of these areas, a paradigm of controlled vibrotactile sensory stimulation was developed (Francis 2000). Using this methodology it is possible to construct brain activation maps of sensory function with high spatial resolution (Gelnar 1998, Maldjian 1999). Using small surface radio-frequency (RF) coils an improvement in signal to noise ratio (SNR) and spatial resolution has been achieved, allowing more detailed investigation of anatomical localisation of activation in the sensory cortex.

We hypothesised that in focal dystonia there are differences in the spatial location and relative orientation of digit tip representation in SI (primary somatosensory cortex). We therefore compared the spatial pattern of the BOLD activity in focal dystonia and control subjects in SI and investigated other cortical areas involved in the sensory perception and processing of vibrotactile stimulation of individual digit tips.

5.2. Methods

5.2.1. Patients and controls

Nine patients and nine control subjects were recruited as described in section 2.8. All of the control subjects were right handed, whilst eight of the patients were right and one left-handed. All the patients recruited to the study had a diagnosis of idiopathic focal dystonia; their diagnosis and symptom duration details are shown in Table 5.1.

<i>Patient</i>	<i>Age</i>	<i>Sex</i>	<i>Dominant Hand</i>	<i>Diagnosis</i>	<i>Symptom duration (yrs)</i>
1	45	M	R	WC	5
2	67	M	R	WC	9
3	69	M	R	WC	12
4	72	M	R	FD	23
5	45	M	R	DC	6
6	35	M	R	WC	3
7	18	M	R	WC	4
8	22	F	L	DC	2
9	45	M	R	WC	8

Table 5.1. Showing the demographic of the patients recruited to the trial. WC – Writer’s Cramp, DC –Dystonic Cramp, FD –Focal Arm Dystonia

Eight of the patients had no other evidence of dystonia elsewhere, while the ninth patient had evidence of dystonia in their left arm, as well as writer's cramp in their

right hand. At the time of scanning, no patients were taking medication or had received botulinum injections in the preceding three months. No subjects had evidence of other neurological or systemic disease. The local ethical committee approved this study and all the subjects gave informed written consent.

5.2.2. Paradigm

Due to the effects of the magnetic field, servo-controlled stimulators are impracticable for fMRI experiments. Piezoelectric bender elements (T220-H4-503 Standard Brass shim Bending element, Piezo Systems, Inc., Cambridge, MA) were therefore used to deliver the mechanical stimulus (a sinusoidal waveform) to the digit tips. A static surround limited the stimulation to a region under the 8 mm diameter Teflon contactor, which was attached to the bender element. In line with the previous investigation on control subjects, an 80 Hz sinusoidal stimulus with peak-to-peak amplitude of 150 μm was employed. Stimulation at this frequency generated robust and repeatable fMRI responses within the post-central gyrus in control subjects (Francis 2000).

Vibrotactile stimulation was applied to both the index (digit 2) and little (digit 5) fingers of the dominant hand in a single scanning session, with the order of stimulation randomised between subjects. Each cycle of the stimulation protocol involved applying the vibrotactile stimulus for 8 seconds, followed by a rest period of 24 seconds, with this cycle being repeated 20 times for each digit. A relatively short 8-second stimulus duration was chosen following its successful use in a previous study (Francis 2000). Reducing the stimulus duration has also been shown to produce better co-localisation of BOLD signal change and elevated neuronal activity (Menon 1999). The bender remained in contact with the finger throughout each experiment and the subjects were instructed to attend to the stimulus.

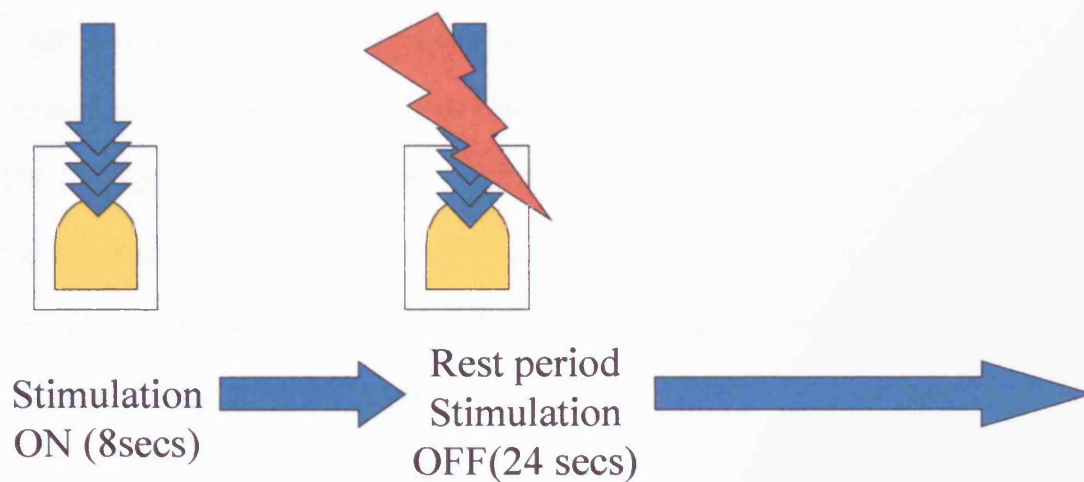


Figure 5.1. Diagram depicting a summary of the paradigm.

5.2.3. Imaging Procedures

Scanning was performed on a purpose built 3.0 T echo-planar imaging (EPI) scanner (Francis 2000), using a 14-cm diameter surface coil placed contralateral to the stimulated digits. Ten contiguous T_2^* -weighted sagittal images with 128 x 128 matrix size, 3 mm in-plane resolution and 4 mm slice thickness were acquired using an MBEST (Modulus Blipped Echo-planar Single pulse Technique) sequence with a 1.9 kHz gradient switching frequency and a 35 ms echo time ($TE = 35ms$). The ten slice capture was performed every 2 s. To help minimise any movement during scanning, all subjects were restrained using inflatable cushioning placed on either side of the head. After the acquisition of the functional data sets, anatomically precise inversion recovery images (with CSF nulled) were acquired using a fast gradient echo technique with resolution of $2 \times 2 \times 1.5 \text{ mm}^3$. These images had the same distortions as the fMRI data, thus allowing precise registration between the functional and the anatomical data sets.

5.2.4. Image Processing

Image analysis was performed using Analyze AVW (Mayo Foundation) and MEDx (Sensor Systems) software. Images were co-registered using a three-dimensional AIR (Automated Image Registration) (Woods 1992) algorithm (6 parameter, rigid body), and then globally normalised. To reduce high frequency noise, the image data were temporally smoothed by convolving with a Gaussian of 3-s full width half maximum. The data were also high pass filtered to 0.01 Hz to remove any low-frequency drift. No spatial smoothing was applied.

5.2.5. Image Analysis

Statistical maps were then generated for each subject, by correlating the time course for each of the pixels with the stimulus waveform convolved with a haemodynamic response function (a Poisson function with a λ -value of 6 s). The resulting statistical maps were thresholded at a corrected probability level of less than 0.005 using cluster detection. To achieve improved 3D representation; the statistical maps were then registered to the corresponding 2 x 2 x 1.5 mm³ resolution anatomical images. The resulting overlay maps then underwent 3D rendering to provide a surface representation of the data.

SI was then approximately separated into cortical subregions (Brodmann areas 3a, 3b, 1, and 2) (Fig. 5.2.) by following the practices of previous investigators (Gelnar 1998). This meant using the anatomy of major sulci and gyri to provide the following operational descriptors. Area 1 was defined as occupying most of the crown of the postcentral gyrus, flanked by areas 2 and 3b located on its posterior and anterior walls respectively. Brodmann's area 3a was defined as occupying the fundus of the central sulcus. Each of the activated clusters within SI were then assigned to fall within these sub-regions of the sensory system.

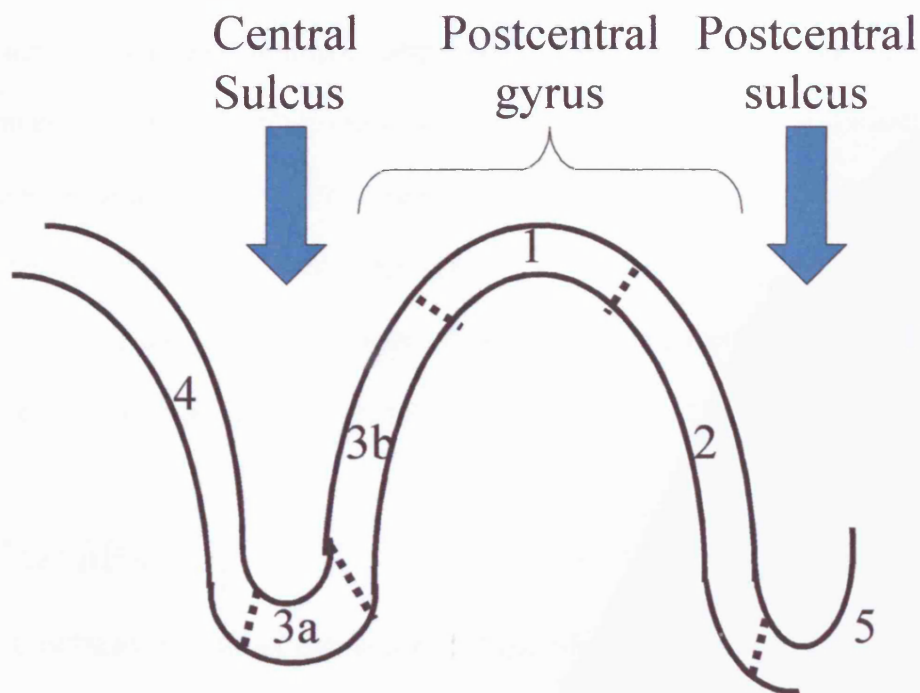


Figure 5.2. Representation of the Separate Brodmann area in the post central gyrus

These activated areas were sampled for the number of significantly activated pixels, average signal percentage change (the average percentage change was computed using the ratio of the average of the maximum of the three points in the time-course relative to the minimum three) and the location of the centre of mass of activation in each individual subject's image space.

Due to the high between-subject and between-hemisphere variability of the curvature of the brain at the resolution at which the fMRI data was acquired, a group analysis has not been performed in this study. Rather, analysis steps have been performed in the individual subjects' image space and statistical tests then performed. Using the centre of activations for the two digits investigated, the distances between representations (anterior/posterior [A/P], medial/lateral [M/L], superior/inferior [S/I] and Euclidean) were calculated for each subject group (patients and controls). To assess the differences in relative location in 3D, a

MANOVA test with differences in three directions as dependent variables was performed accounting for multiple comparisons. If the 3D vector showed significant differences, a t-test was employed to investigate the significance of separation of digit representation in each of the three orthogonal directions (accounting for any sign changes indicating reversed ordering).

Significant differences in the number of pixels and percentage signal changes of digits were assessed using an independent t-test.

5.3. Results

5.3.1. Cortical Areas Activated by Digit Stimulation

Vibrotactile stimulation of individual digit tips (digits 2 and 5) resulted in significant activation in the primary sensory cortex, secondary somatosensory cortex (SII/area 40), posterior insula, and posterior parietal cortex for both the control and patient groups. There was no significant difference in those areas found to be activated between the two subject groups, as indicated in Table 5.2. However, significant differences were found in both the spatial pattern and strength of this activation. Figure 5.3. shows representative data of a statistical map overlaid on an individual subject's anatomical images.

	<i>Area 1</i>	<i>Area 3b</i>	<i>Area 40</i>	<i>Area 5</i>	<i>Ant. Insula</i>
Digit 2 <i>Controls</i>	9	9	9	9	8
<i>Dystonics</i>	8	8	9	8	6
Digit 5 <i>Controls</i>	9	9	9	9	7
<i>Dystonics</i>	9	9	9	7	5

Table 5.2. Number of subjects showing activation within identified areas following stimulation of digits 2 and 5.

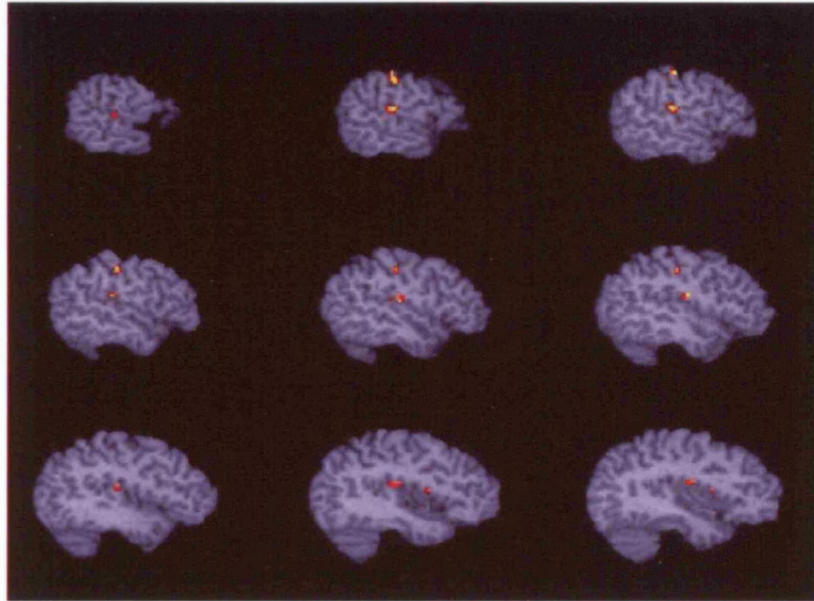


Figure 5.3. Representative statistical maps from the control group during stimulation of digit two only.

5.3.2. Three Dimensional Separation of Digit Cortical Representations.

Table 3. Shows the absolute separation of representations, both in 3D and each of the planes (Anterior/posterior (A/P), Medial/lateral (M/L), Superior/Inferior (S/I)), between sites of activation due to stimulation of digits 2 and 5 in the two subject groups. There was a significant difference in the absolute 3D separation in area 1 of SI between the control and dystonic groups ($p < 0.001$), and this is illustrated in Figure 5.4.a (controls) and b (patients), using data from two representative subjects. A breakdown of the 3D separation into individual vectors revealed significant differences between the control and the dystonic groups in area 1 in all three directions (A/P – $p < 0.001$, M/L – $p < 0.001$, S/I – $p = 0.02$), with the ordering being reversed in both the anterior/posterior and superior/inferior directions. There was no significant difference in the 3D-digit separation in the other areas. However, a strong trend can be seen in Area 40 (Figure 5.5.a and b) for a reversal of ordering of activation in the anterior/posterior direction. Area 5 also revealed a strong trend for reversed ordering of digits in the medial/lateral direction.

Area	3D separation		Anterior/Posterior		Medial/Lateral		Superior/Inferior	
	Dystonia	Controls	Dystonia	Controls	Dystonia	Controls	Dystonia	Controls
1	4.14 (0.23)	9.6 (1.24)	0.75 (0.75)	-4.95 (0.89)	0.7 (0.82)	5.91(0.71)	-0.38 (1.05)	4.09 (1.58)
3b	2.17 (0.65)	2.63 (0.68)	1.13 (0.55)	-0.61 (0.80)	0.03 (0.54)	0.37 (0.63)	0.75 (0.49)	0.61 (0.37)
SII/Area 40	2.89 (0.60)	4.04 (0.87)	1.00 (0.92)	-1.90 (0.97)	0.53 (0.61)	-0.58 (0.73)	-0.33 (0.35)	0.24 (0.94)
Posterior Parietal	2.33 (0.54)	1.84 (0.32)	0.00 (0.61)	-0.33 (0.40)	0.74 (0.49)	-0.94 (0.44)	0.43 (0.61)	-0.67 (0.15)
Insula	2.68 (0.82)	3.35 (0.43)	0.60 (0.47)	-1.29 (0.71)	-0.48 (0.34)	-0.90 (0.63)	0.60 (1.16)	0.71 (0.74)

Table 5.3. Separation in mm of digit 2 and digit 5 representations in the two groups. The three dimensional and individual Cartesian components of the separation are listed (standard deviations given in brackets).

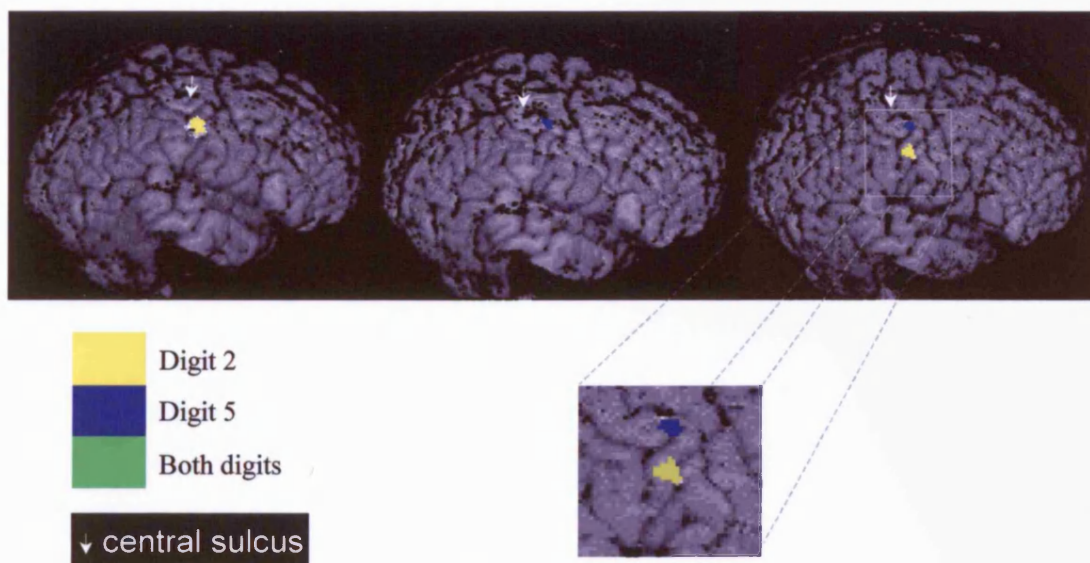


Figure 5.4.a Findings in Area 1 for representative subjects from the control group. Activation resulting from digit 2 tip stimulation only is shown in yellow, that from stimulation of digit 5 tip only in blue, while areas activated by stimulation of both digit tips are shown in green.

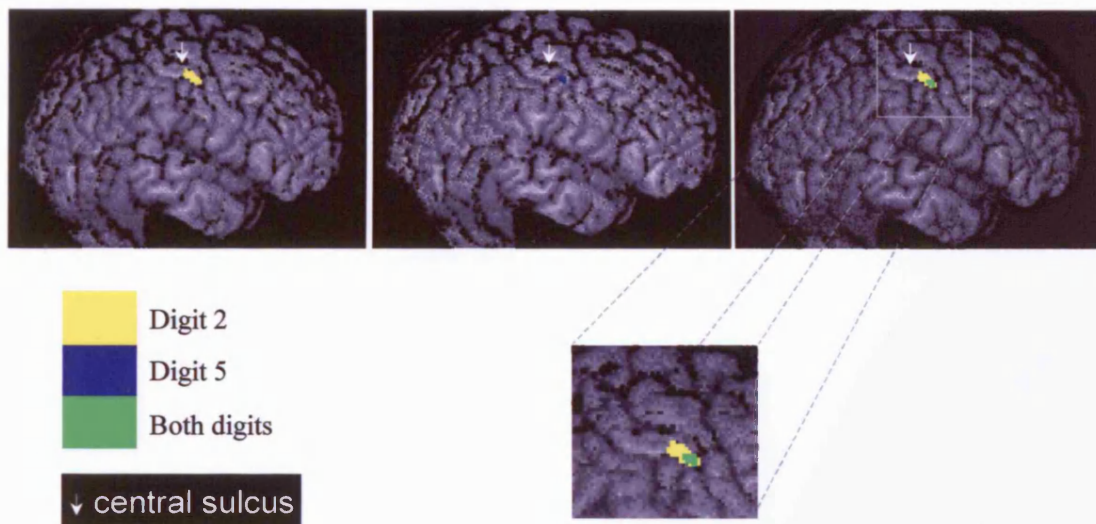


Figure 5.4b. Findings in Area 1 for representative subjects from the dystonic patients. Activation resulting from digit 2 tip stimulation only is shown in yellow, that from stimulation of digit 5 tip only in blue, while areas activated by stimulation of both digit tips are shown in green.

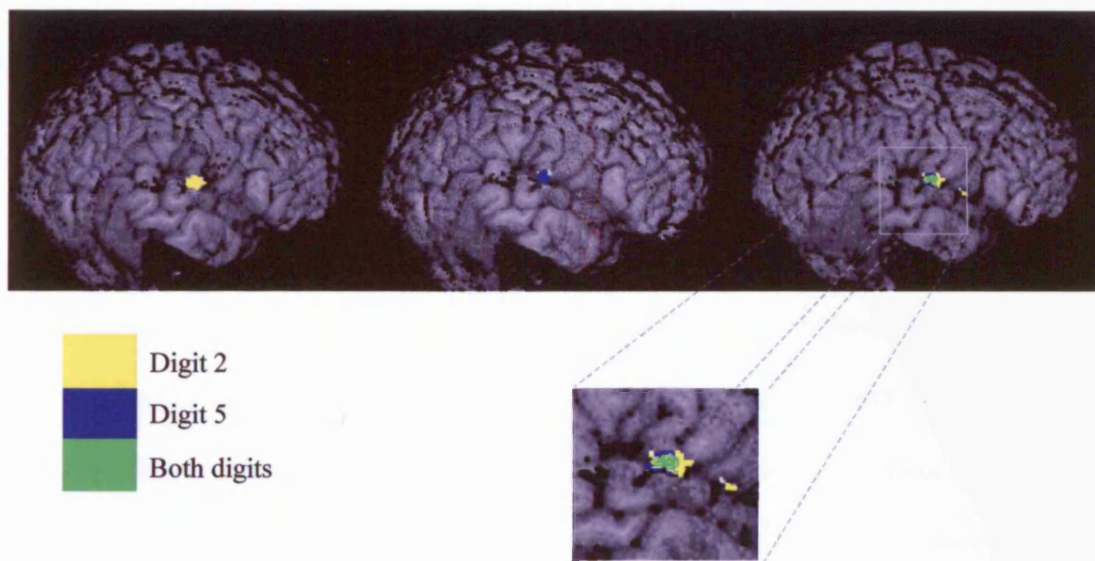


Figure 5.5a. Findings in Area 40 for representative subjects of the control group. Activation resulting from digit 2 tip stimulation only is shown in yellow, that from stimulation of digit 5 tip only in blue, while areas activated by stimulation of both digit tips are shown in green.

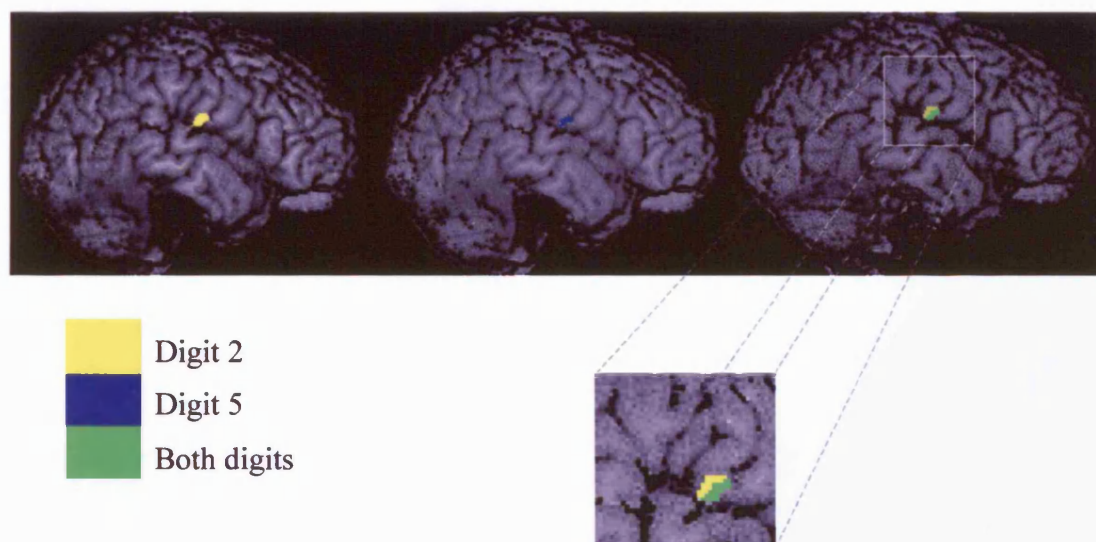


Figure 5.5b. Findings in Area 40 for representative subjects the dystonic patients. Activation resulting from digit 2 tip stimulation only is shown in yellow, that from stimulation of digit 5 tip only in blue, while areas activated by stimulation of both digit tips are shown in green.

5.3.3. Extent and Percentage Change of Activations

Extent of activation, as assessed by the number of pixels in an area at a corrected probability threshold of less than 0.005, also revealed significant differences between the two subject groups. Table 4 shows the number of pixels and percentage signal changes for stimulation of digit 2 in both subject groups and Table 5 shows the same information for digit 5. Stimulation of digit 2 generated a smaller extent of activity (expressed in terms of number of pixels) in the secondary sensory cortex ($p < 0.004$) and the posterior parietal areas ($p < 0.0002$) in the dystonic group compared with the control group. Stimulation of digit 5 showed significant under-activity only in the secondary sensory cortex ($p < 0.035$). For both digits studied, there were no significant differences in the percentage signal change measured in activated areas between dystonic and control groups.

Area	Number of Pixels			Percentage Change		
	Dystonia	Controls	p-value	Dystonia	Controls	p-value
1	5.50 (1.00)	7.88 (0.90)	NS	2.61 (0.21)	3.19 (0.33)	NS
3b	2.25 (0.59)	2.00 (0.47)	NS	2.28 (0.40)	2.00 (0.54)	NS
SII/Area 40	3.33 (0.67)	8.33 (1.24)	<0.005	2.18 (0.32)	1.96 (0.21)	NS
Posterior Parietal	2.25 (0.25)	6.56 (0.71)	<0.005	2.50 (0.35)	2.73 (0.43)	NS
Insula	1.44 (0.47)	2.22 (0.55)	NS	2.30 (0.33)	2.09 (0.41)	NS

Table 5.4. Number of pixels and average percentage change in activated areas for stimulation of digit 2 (Standard deviations given in brackets), plus independent t-test results. (NS) refers to not significant.

Area	Number of Pixels			Percentage Change		
	Dystonia	Controls	p-value	Dystonia	Controls	p-value
1	3.11 (0.79)	4.33 (0.29)	NS	2.32 (0.30)	1.98 (0.26)	NS
3b	1.89 (0.45)	1.22 (0.36)	NS	1.30 (0.25)	1.28 (0.35)	NS
SII/Area 40	2.88 (0.72)	6.00 (1.12)	0.035	2.25 (0.25)	1.88 (0.16)	NS
Posterior Parietal	2.00 (0.53)	3.78 (1.09)	NS	1.50 (0.32)	2.10 (0.98)	NS
Insula	0.88 (0.35)	2.33 (0.62)	NS	1.63 (0.37)	1.82 (0.16)	NS

Table 5.5. Number of pixels and average percentage change in activated areas for stimulation of digit 5 (Standard deviations given in brackets), plus independent t-test results. (NS) refers to not significant.

5.4. Discussion

The results of this study are consistent with the hypothesis that in focal dystonia there are differences in the spatial location and relative orientation of digit tip representation in SI shown by significant differences in both absolute 3D separation and individual vectors. This finding adds more evidence to our emerging understanding that the sensory system is disturbed in focal dystonia. This abnormal representation of the digits as revealed by peripheral sensory stimulation is not a new finding. The intracortical distance between the representation of the second and fifth digits has previously been shown to be reduced in patients with dystonia. Our findings of a mean separation between digits 2 and 5 of 9.60 ± 1.2 mm for the control group and 4.1 ± 0.2 mm for the dystonic group concur well with an electrophysiological study using somatosensory evoked potentials (SSEP's) (Bara-Jimenez 1998). This showed a cortical separation between the second and fifth digit representations of 12.7 ± 5.7 mm in control subjects and 6.5 ± 3.0 mm in the dystonic group in SI. A further study using magnetic source imaging examined the medial/lateral extent covered by all the digital representations in area 3b of SI (expressed as the digit representation fusion index) and found this to be reduced in dystonic subjects (Elbert 1998). This again suggests that digital representations are compressed and disordered in dystonia. We have also shown consistent overlap and occasional inversion of digit representations.

Although the degree of disorganisation of digital representations identified here in patients with focal dystonia concurs with previous studies, previous work in dystonia has demonstrated sensory abnormalities in area 3b of the primary somatosensory cortex. Investigations on the owl monkey, which provides an animal model for focal dystonia, showed a fusion of representations in area 3b (Byl 1996),

and postulated that this area has an influence on motor control via five principle areas, namely 1, 2, 3a, 5, SII. Follow up work on dystonic patients at the time of stereotactic surgery (Lenz 1999) additionally showed receptive field changes in the ventral caudal nucleus of thalamus, a portion of the thalamus thought to project mainly to area 3b, but also to area 1 (Kandel 2000).

This present study reveals the siting of major abnormalities in the primary somatosensory cortex (SI) occur primarily in presumptive area 1, occupying the crown of the post central gyrus. Indeed, it was difficult to detect digit separation in area 3b even in control subjects, rendering the measurement of possible abnormalities in the patient group impossible. Previous fMRI studies using vibrotactile stimuli to map the somatosensory cortex of normal subjects have also failed to show a clear separation of digit representation in area 3b (Francis 2000, McGonigle 1998). A previous study using a low frequency vibrotactile stimulus did not separate SI into its subregions (Maldjian 1999). However, those fMRI studies exploring somatotopy of the hand using electrical stimulation, have revealed a large separation of digit representation in area 3b. One investigator reporting a separation of digits 1 and 5 on the order of 16 ± 5 mm in area 3b, compared to a smaller highly variable separation of 14 ± 11 mm in area 1 (Kurth 1998, Kurth 2000). Krause *et al* extended such studies using electrical stimulation to investigate the influence of stimulus intensity on somatotopy. They found that an increase in stimulus intensity applied to individual digits leads to a larger relative overlap of activation sites in area 1 and 3b, this increased overlap being greatest in area 1 (Krause 2001). Magnetic source studies, selectively sensitive to those tangential source signals (area 3b), and relatively insensitive to radial sources (area 1), also reveal strong separation

of digits in area 3b, of the order of 15 mm for digit 1 to 5 separation (Baumgartner 1991, Tecchio 1997).

There are several potential reasons why this current study was insensitive to digital separation in area 3b. Firstly, there are methodological considerations, of which the mode of applied stimulation applied is of particular importance. As described above, electrical nerve stimulation appears to generate more discernible area 3b activation and larger digit separation than vibrotactile stimulation. Secondly, the frequency of vibrotactile stimulation is of importance. We have found that the functional maps show differential activation patterns depending on the frequency of stimulation, SI being more active at lower frequencies and SII at high (Francis 2000). There may indeed be some organisation of frequency dependence between area 3b and area 1 related to the receptor afferent. Also, somatosensory cortical responses have complex spatio-temporal dynamics which are highly dependent on stimulus (Lee 1992, Nicolelis 1995). Finally, there are anatomical considerations regarding the location of area 3b. This area is located deep in the post-central sulcus, which may make separation more difficult to discern than in that of area 1 on the crown of the gyrus. Cortical flattening techniques (Carmen 1995) may allow better visualisation of separations in area 3b and is a subject for further studies.

Analysis of the sensory activation in the control group revealed somatotopic organisation in the secondary sensory cortex (SII/Brodmann area 40), with the area activated by stimulation of digit 5 being more posterior than that of digit 2. This is in accordance with previous neurophysiological studies (Krubitzer 1995), receptive field mapping (Burton 1986), MEG (Maeda 1999) and fMRI (Francis 2000) studies. This anterior/posterior organisation revealed a trend for reordering in the dystonic group. The activity in this area (indicated by the number of activated pixels) was

also found to be significantly reduced during digit stimulation of both digits 2 and 5. However the percentage change was not significantly reduced, suggesting a more focal high level response in dystonics. The functional significance of SII in humans has not yet been fully elucidated. There is evidence from lesion studies in both monkeys (Ridley 1978) and humans (Caselli 1993), that SII has roles in sensory learning and movement control especially in skilled movements that require sensory feedback (Huttunen 1996). Human studies have additionally demonstrated enhancement of SII activity during movement (Huttunen 1996, Forss 1998) and disruption of movement by direct stimulation of SII (Penfield 1954). These findings indicate that SII may have a role in the integration of sensory information into motor programmes.

Clearly, one possible explanation for the disrupted activity of SII in dystonic patients is that it is consequential of the abnormal activation in SI. However, the exact functional relationship of cortical sensory areas SI and SII remains controversial with considerable debate over whether SI and SII are ordered via a serial or parallel process (Hari 1999). Lesion studies in primates and other animals (Rowe 1996, Pons 1987, Pons 1992) as well as SEP (Lunders 1985), MEG (Mauguierere 1997) and PET (Paulescu 1997) studies in humans have so far been unable to conclusively demonstrate the nature of the processing relationship between SI and SII relationship. Clearly, abnormal SII activity in dystonia may be a reflection of disordered input to this area from either SI or the thalamic region. There also remains the possibility that there is in fact an intrinsic abnormality of SII that is itself responsible for its abnormal activity.

In addition to the digit organisation and activity abnormalities of SI and SII, the present study also demonstrated less pronounced abnormalities in the posterior

parietal cortex (area 5). This area was found to be significantly under active (indicated by the reduced number of activated pixels) for digit 2 stimulation only (although digit 5 stimulation did also show a trend toward being under active). It may be that this disparity reflects the dystonia, this finger being more affected in the cases studied, but it is hard to draw any firm conclusions from this. As in SII, no significant difference in percentage change was found between dystonic and control groups.

The abnormalities in dystonia during the integration of sensory information into motor programmes and the associated abnormalities in motor output is clearly beyond the scope of this study. However, this study demonstrates a number of abnormalities at different levels of sensory processing. Further work looking at the dynamic changes of these abnormalities during movement and the interplay between these and disordered motor output is planned.

Although a number of sensory abnormalities have now been shown to be present in dystonia, they are clearly not the whole story. Cortical activation studies using PET have demonstrated many other distributed areas of under and over-activity in patients with dystonia. It is also noteworthy that Braille readers (a group who do not seem to have an increased risk of dystonia) also display similar findings of ‘smearing’ of digital representations in the primary sensory cortex (Sterr 1998).

The possibility remains that the sensory changes observed in this and other studies are actually secondary to the dystonia, rather than causative. However, whilst an abnormality in motor response following a movement task could easily explain widespread under/over activity of the sensory cortex, this does not explain alterations in the cortical location following single digit stimulation. If it were possible to perform this study on a group of pre-symptomatic individuals, this may

allow us to answer the question of whether altered sensory processing causes dystonia, or vice-versa.

5.5. Conclusion

This study has demonstrated an abnormal arrangement in the cortical sensory representation of digits 2 and 5 in area 1 of the somatosensory cortex. It has also provided some evidence that there is a more widespread sensory processing abnormality in focal dystonia.

Chapter Six

Pilot Investigations into the Intracortical Organisation of Discrete Movements in Normal Individuals and Those with Focal Dystonia.

6.1. Introduction

In light of the findings of the study presented in Chapter 5 and other studies showing an abnormal organisation of the sensory cortex in dystonia, hypotheses were developed as to how this apparent abnormal sensory functional organisation could give rise to the abnormal motor sequencing that characterises the dystonic contractions. One such hypothesis postulated that there maybe a similar abnormal organisation within the motor cortex mirroring that of the sensory cortex. This hypothesis is attractive, for if such organisational upset were to exist in the motor system, it would be easy to explain the concomitant activation muscles in dystonia.

Somatotopic mapping and assigning of functions to the motor cortex has historically been a fruitful area of research. The advent of functional imaging has increased the interest in this area further.

Originally it was thought that any part of the brain could control movement (Flourens 1842, Lashley 1929). However, it soon became clear that the motor cortex dominated

the control of movement and that it had a functional organisation (Vogt 1919, Foerster 1936). Penfield and Boldrey's investigations into this organisation were published in 1937. Their paper presented the results of cortical electrical exploration of 163 patients at the time of surgery. They proposed that the motor cortex had a somatotopic organisation (the motor homunculus), a concept that remained unchallenged for many years (Penfield 1937). The motor homunculus is a 'distorted cartoon of the body', a continuous representation of the movement of the body from foot movements at the vertex of the motor strip to tongue and pharyngeal movements at its lower extremity. These findings seem to correlate well with the known clinical phenomenon of the epileptic 'Jacksonian march' (Jackson 1931).

As techniques became more refined, it became clear that the organisation of the motor cortex was more complex. Penfield detailed the wide distribution of stimulation points resulting in movements of the same finger and the fact that discrete stimulations often resulted in compound movements of a number of fingers. He also detailed the considerable variability of individuals (Penfield 1950).

In more recent times, functional imaging (PET, MEG and fMRI) has been used in humans as a non-invasive tool to investigate the spatial distribution of finger motor representations. The results have been contradictory, with some investigators claiming demonstrations of somatotopy (Cheyne 1991, Grafton 1993, Kleinschmidt 1997, Lotze 2000, Beisteiner 2001, Indovina 2001, Hlustik 2001) and other demonstrating overlapping finger representations (Rao 1995, Sanes 1995, Dechent 2003, Sanes 2000, Schieber 2001, Sanes 2001). The more recent studies suggest a degree of finger somatotopy especially when patients are imaged at high field with spatial high resolution (Beisteiner 2001, Indovina 2001).

Theoretically, when considering motor cortical organisation in dystonia, there aren't just issues relating to the separation of individual digits: the problem is more complex. Co-contraction may represent abnormal somatotopy at a different level entirely, that of agonist versus antagonist within a somatotopic area. This degree of somatotopy has been explored during movements around large joints (Colebatch 1991) but not in smaller joints.

It was therefore decided to engage in two pilot studies using 'paradigm one' to investigate the possible motor somatotopy of finger flexion and extension in control subjects and 'paradigm two' to investigate the hypothesised derangement of individual finger motor cortical representations in patients with focal dystonia.

6.2. Methods

6.2.1. Patients and Controls

Five control subjects were recruited to the study using 'paradigm one' (see section 6.2.2.1.) following the recruitment policy outlined in section 2.8. Four control subjects were right handed and one left handed with an average age of 25 ± 5.2 .

Three control and three dystonic subjects were recruited to the study using 'paradigm two' (see section 6.2.2.2.). The control subjects had a mean age of 32 ± 4.6 and the patients a mean age of 36 ± 7.2 . All the control subjects were right handed. The details of the patients are displayed in Table 6.1.

<i>Patient Number</i>	<i>Age</i>	<i>Sex</i>	<i>Dominant Hand</i>	<i>Affected Hand</i>	<i>Dystonia Type</i>
1	28	F	L	L	OD (t)
2	42	F	R	R	OD (t)
3	38	M	R	R	OD (g)

Table 6.1. Patient Data (OD- occupational dystonia, (t) typists dystonia, (g) guitar players dystonia)

6.2.2. Paradigms

6.2.2.1. Paradigm 1

In order to localise the motor cortical somatotopic representation of individual muscle movements around a single distal joint, a paradigm involving flexion and extension around terminal phalanx of the index finger was devised. The index finger was chosen as it is often implicated in the dystonic cramps displayed by patients.

In order to achieve clarity within the paradigm, movements except those of the finger have to be minimised. This was achieved by attaching the subjects arm and hand to a board with a space free for the movement of the terminal parts of the index finger. The patients were then visually cued a ‘task encoding’ stimulus as to which direction the index finger should be moved (flexion ‘5’, extension ‘2’). This stimulus was presented in a pseudo-random order consisting of 20 of each type of stimulus. Simultaneous with the task information, a task duration stimulus (a green bar) was shown to define the length of the task, this stimulus was present for 8 seconds. This was then followed by a rest period, which was 24 seconds in duration. The total paradigm length was approximately 25 minutes. A summary of this paradigm is shown in Figure 6.1.

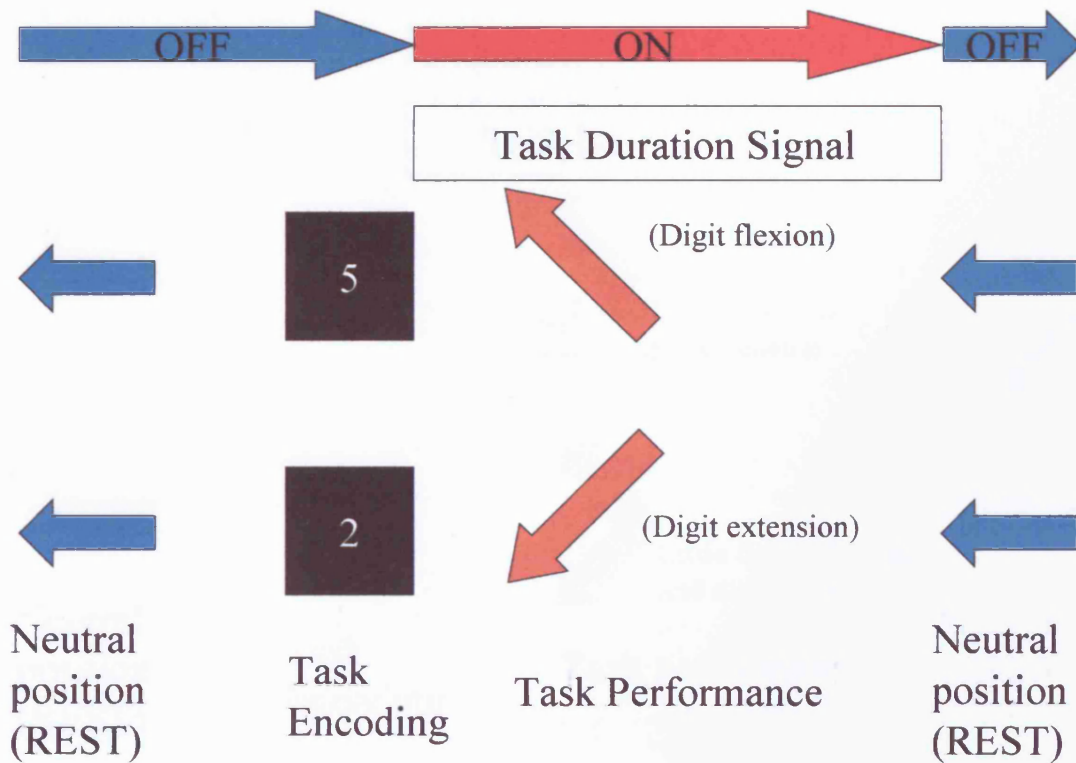


Figure 6.1. Summary of paradigm One

6.2.2.2. Paradigm 2

In addition to paradigm one, another paradigm was developed to look at the spatial cortical mapping of individualised digit movements. The movements in separate digits (thumb (digit 1) and the little finger (digit 5)) were generated by repeated button pressing. A similar system to that used in paradigm 1 was employed to minimise other arm movements. The paradigm design and timings were also similar except for the visual cueing. Here the number '1' cued movements of the thumb and '5' cued movements of the little finger. A summary of this paradigm can be seen in Figure 6.2.

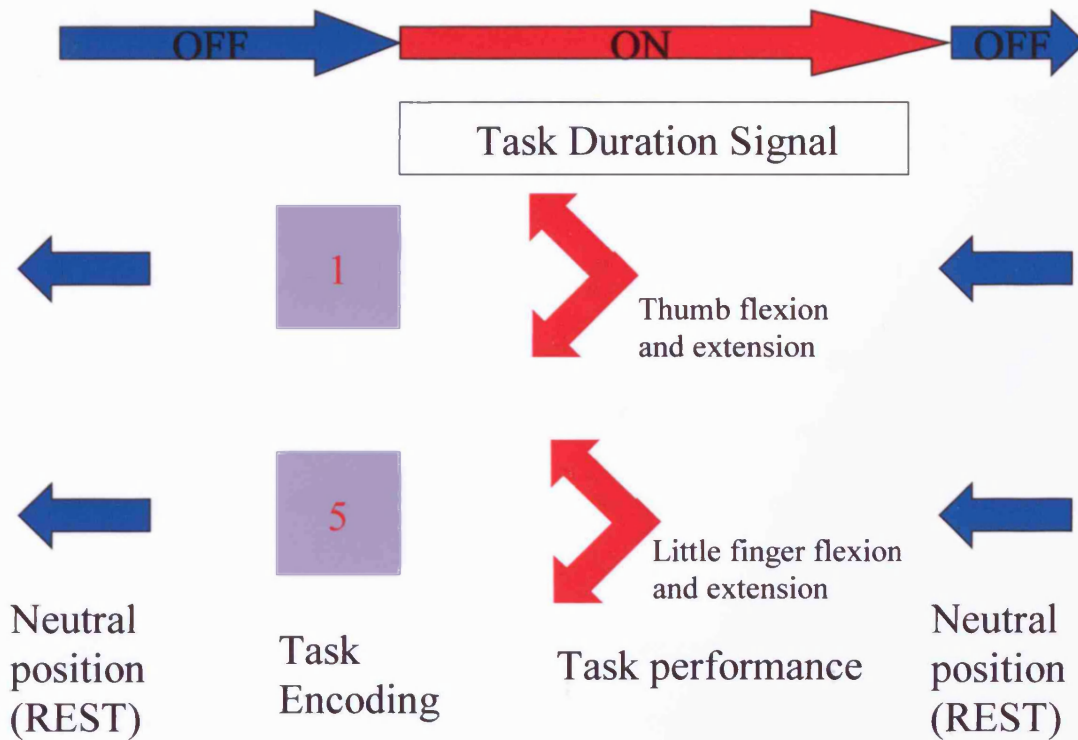


Figure 6.2. Summary of paradigm two.

6.2.3. Imaging Procedures Imaging procedures

The imaging procedures undertaken were the same as those described in Section 5.2.3. High resolution imaging of 2mm X 2mm X 4mm were used for all paradigms. See Figure.6.3. for a summary of the imaging procedure.

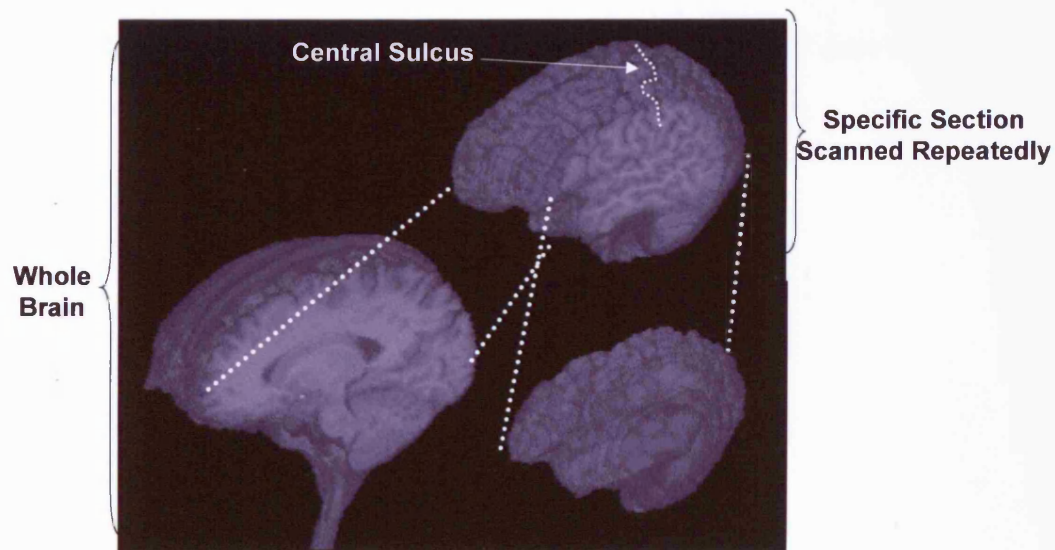


Figure 6.3. Summary of imaging procedure

6.2.4. Image Processing

The image processing undertaken for both paradigms was the same as described in section 5.2.4.

6.2.5. Image Analysis

Activation of areas outside the motor cortex was noted, but no detailed analysis was made of any active areas other than those within the motor cortex. Assessment of 3D separation of cortical activation was undertaken for both paradigms as described in section 5.2.5.

In paradigm 2, an additional spatial activation analysis was made within the motor cortex following the same methods described in section 4.2.5.3.

6.3. Results

6.3.1. Paradigm No 1.

6.3.1.1. Activation

Reproducible activation of the motor cortex was seen with this paradigm in all the study subjects. Activation in the lateral premotor cortex and the sensory cortex was also noted. A representative activation overlay map is shown in Figure 6.4.

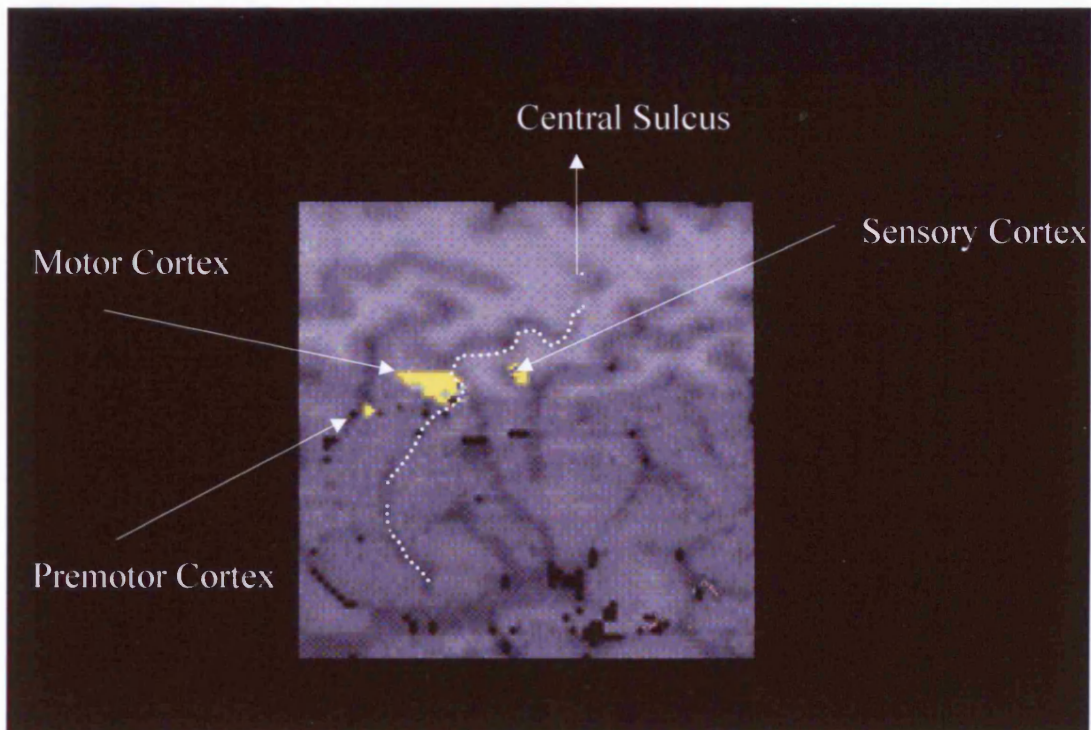


Figure 6.4. Overlay map of Index finger flexion (paradigm one). Active areas at $p < 0.005$

6.3.1.2. Three Dimensional Separation of Digit Cortical Representations.

Figure 6.5. shows the double overlay map containing representations of flexion and extension of the index finger. As these pictures indicate, the activations are totally contained within each other. The 3D separation of the centres of activity was less than 1mm. This was a repeatable finding amongst the control subjects studied, although the location of the activated area with the motor cortex varied slightly.

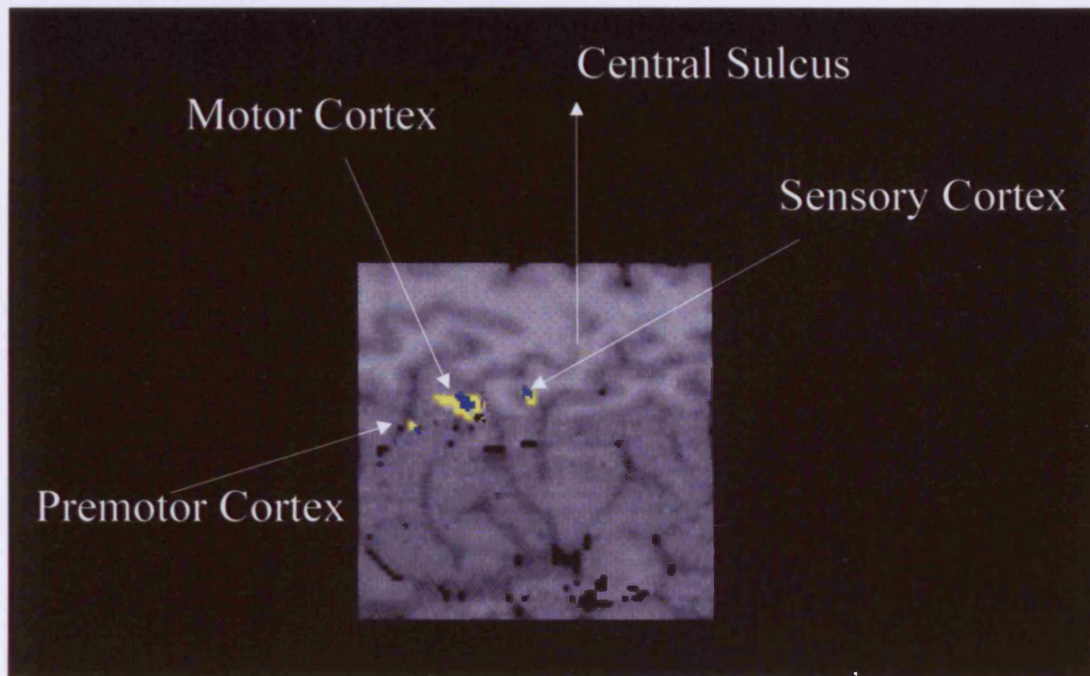


Figure 6.5. Double overlay of Index finger flexion (yellow) and extension (Blue)
.Active areas at $p < 0.005$.

6.3.2. Paradigm No 2.

6.3.2.1. Activation

Both groups showed activation in the motor cortex, lateral premotor cortex, sensory cortex. Brodmann area 43 activation and posterior parietal activation was seen individuals of both groups but not in all subjects. The same areas were activated by both thumb and little finger movements. The figures below display the areas activated in control subjects (Fig.6.6) and dystonic subjects (Fig.6.7). A summary of areas activated is displayed in Table 6.2 with percentage change displayed in Table 6.3.

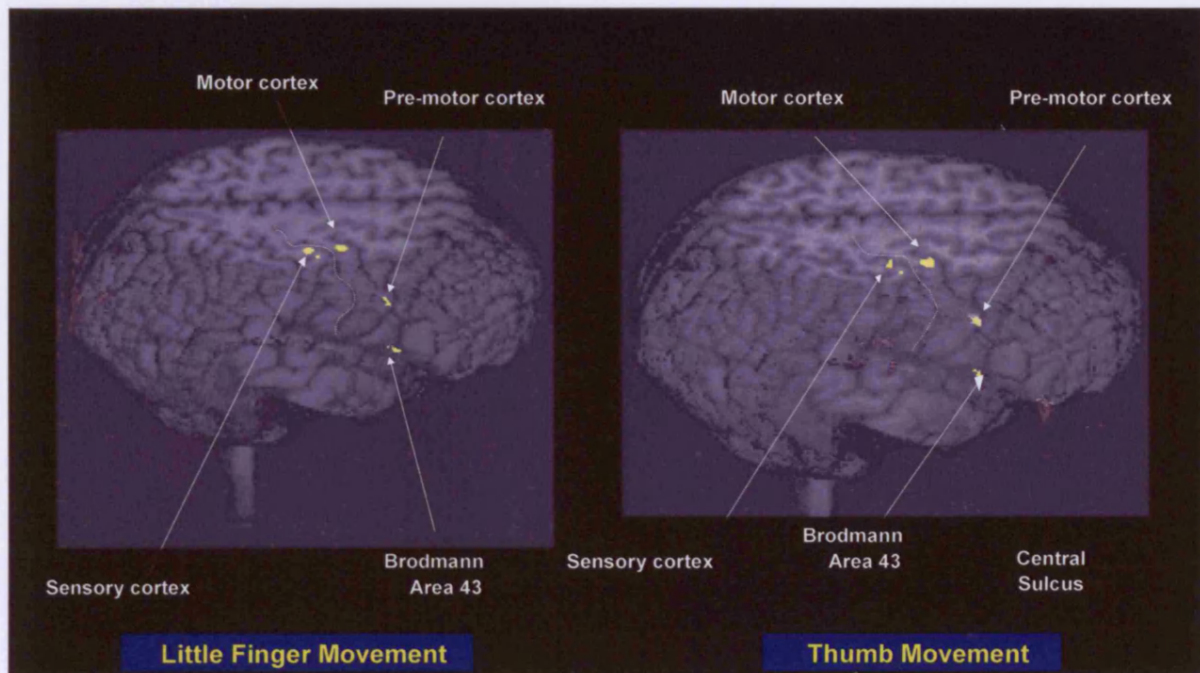


Figure 6.6. Overlay maps for control subjects. Active areas $p < 0.005$

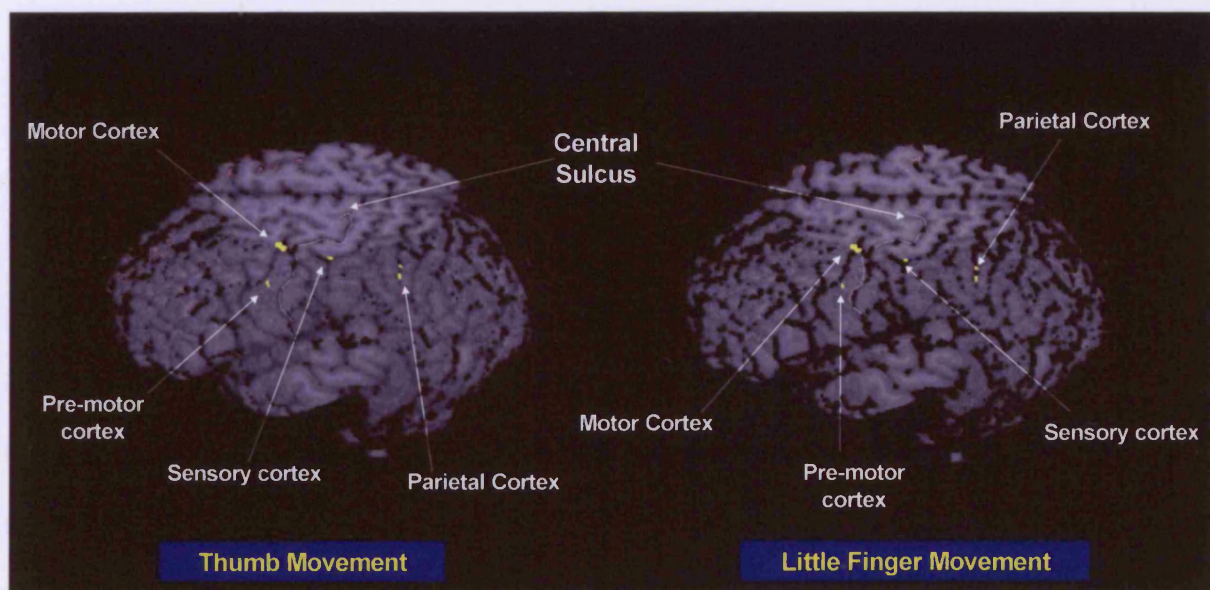


Figure 6.7. Overlay maps for dystonic subjects. Active areas $p < 0.005$

<i>Area</i>	<i>Controls</i>		<i>Dystonics</i>	
	<i>Pixels</i>	<i>%change</i>	<i>Pixels</i>	<i>% change</i>
Motor cortex	8.0	2.8	6.3	2.4
Sensory cortex	6.6	2.4	6.3	2.1
Premotor cortex	5.3	2.3	5.3	2.1
Parietal cortex	3.6	1.1	2.6	0.8
Brodman 43	1.0	1.4	2.0	1.6

Table 6.2. The percentage change and number of pixels activated during thumb movements

<i>Area</i>	<i>Controls</i>		<i>Dystonics</i>	
	<i>Pixels</i>	<i>%change</i>	<i>Pixels</i>	<i>% change</i>
Motor cortex	8.3	2.4	6.3	2.7
Sensory cortex	4.6	2.3	4.3	2.2
Premotor cortex	5.3	2.3	3.3	1.8
Parietal cortex	not active	not active	2.6	1.3
Brodman 43	1.3	0.9	1.0	0.7

Table 6.3. The percentage change and number of pixels activated during little finger movements

6.3.2.2. 3D Separation of Digit Cortical Representations

As shown in Figures 6.6 and 6.7, the motor representation of these movements in both control and dystonic subjects is almost identical and overlapping. (Fig 6.8.) After transformation to Talairach co-ordinates, a 3D separation of less than 1mm between thumb and little finger was found for both control and dystonic subjects in all the activated areas.

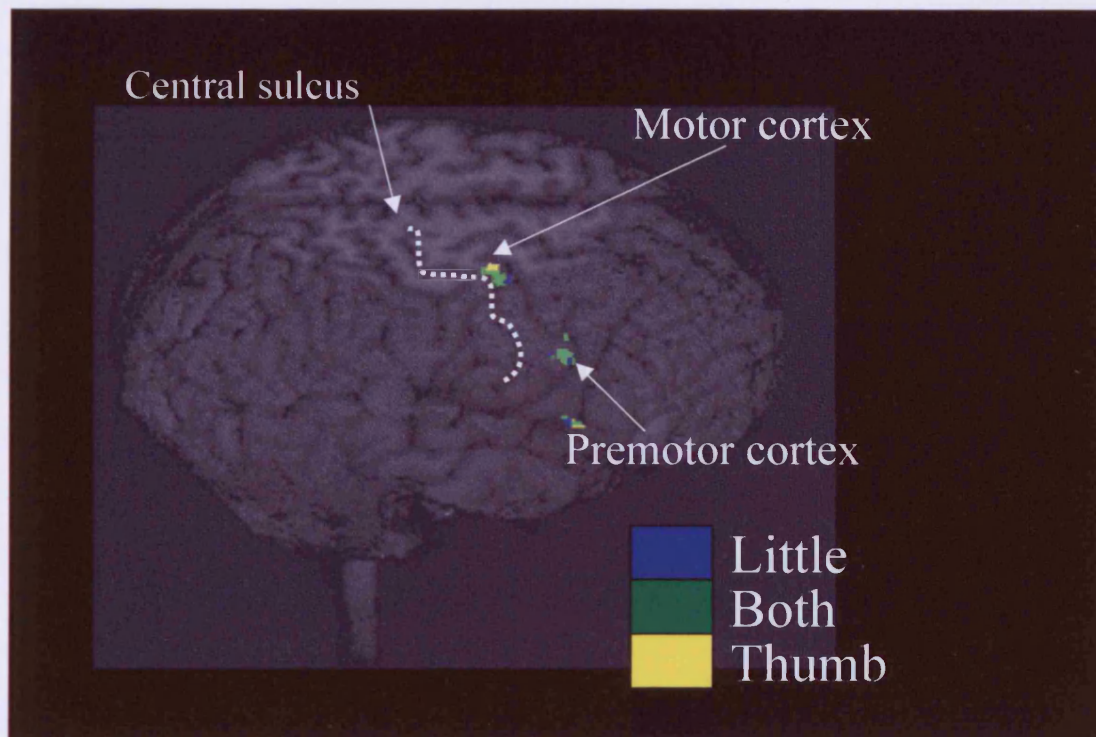


Figure 6.8. Double overlay of thumb and little finger representations in control subjects

6.3.2.3. Motor Cortex Activation

A composite of the total activity displayed by the motor cortex time course for all the control subjects and dystonic patients is shown in Figure.6.9a (thumb) and Figure 6.9b. (little finger). There is quantitatively a decrease in the peak activation of the motor cortex during thumb movement in the dystonic group, but this does not show any statistical significance. Motor cortex activity for little finger activation is similar in both groups.

6.3.2.4. Motor Cortex Region Analysis

The region of the motor cortex that is responsible for the motor representation of the thumb, little finger, and middle finger is shown in Figure 6.10.

Figure 6.10. A map of the motor cortex showing the representation of the thumb, little finger, and middle finger.

Figure 6.10. A map of the motor cortex showing the representation of the thumb, little finger, and middle finger.

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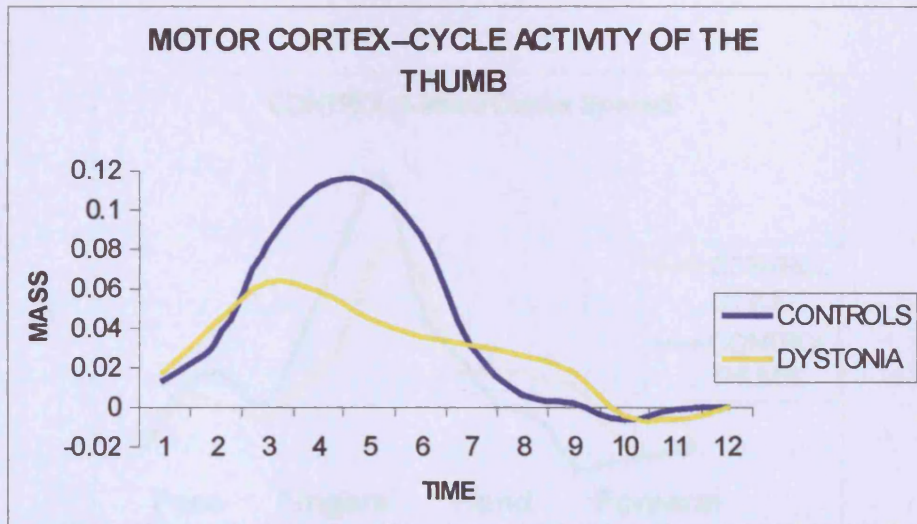


Figure 6.9a. Activity of motor cortex across paradigm – Thumb

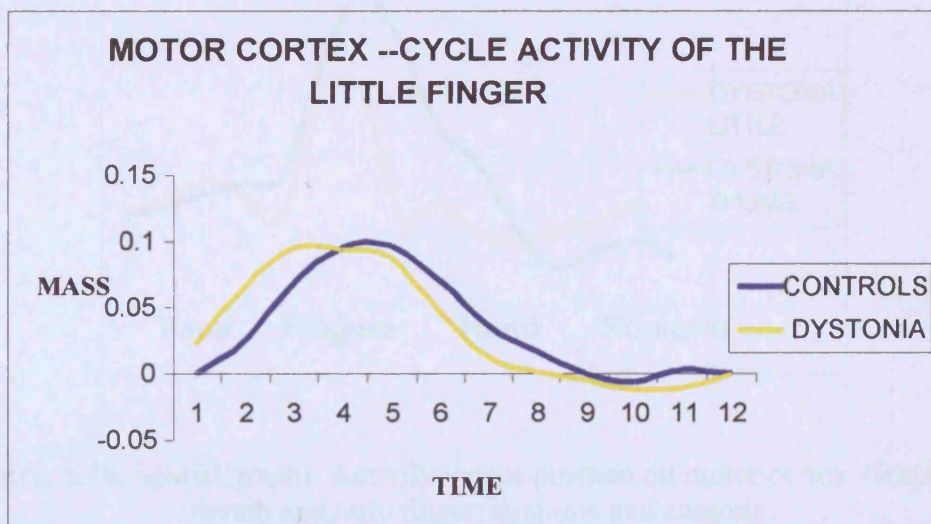


Figure 6.9b. Activity of motor cortex across paradigm – Little Finger

6.3.2.4. Motor Cortex Spread Analysis.

No significant difference in the spatial activation of the motor cortex was seen. There was, however, a trend for the activation of the thumb in the dystonic subjects to be more extensive. Figure 6.10. displays a composite of the spread of activity for both fingers of both groups.

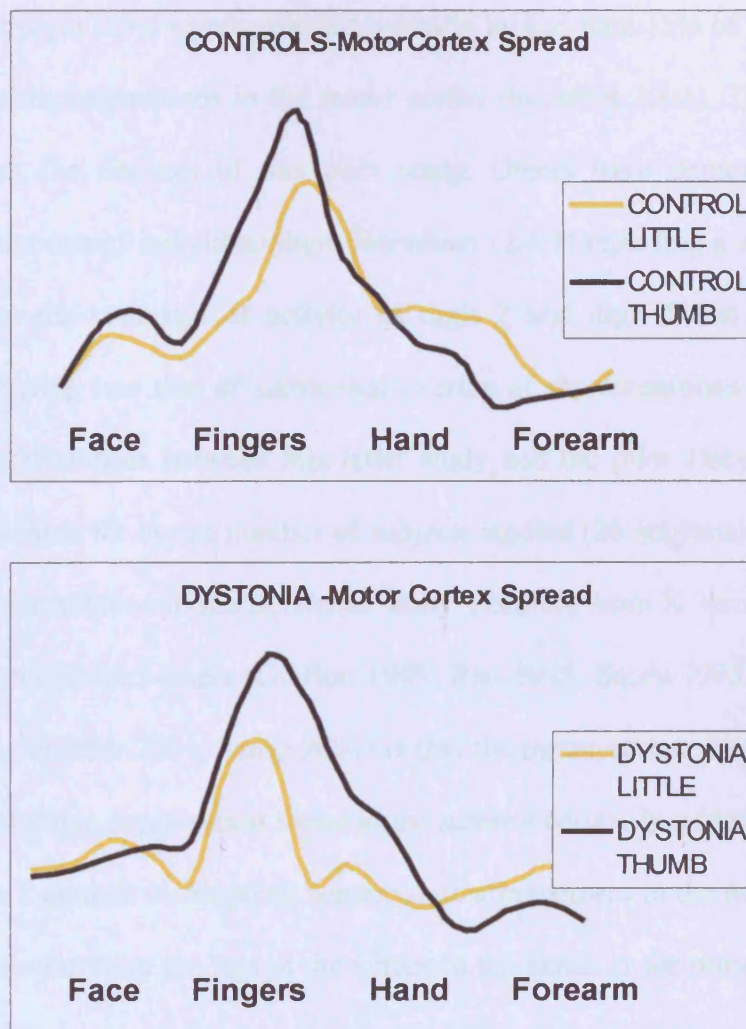


Figure. 6.10. Spatial graphs. Activity versus position on motor cortex. Graphs for thumb and little finger, dystonia and controls

6.4. Discussion

The inability of these two pilot investigations to discriminate a clear and separate motor homunculus in control subjects precluded further investigation into the possible abnormalities in dystonia. Although these two pilot investigations were negative and contained too few subjects for meaningful analysis, they do throw some light into the functioning of the motor cortex and its organisation.

In a similar study to the pilot study presented above it was shown that the centre of activation of digits 2,3,4 were separated typically by less than 15% of the entire extent of the digital representations in the motor cortex (Indovina 2001). This finding is in keeping with the findings of this pilot study. Others have demonstrated slightly greater separations of individual digit, Beisteiner (2001) reporting a separation of 2.4 mm between the centroids of activity of digit 2 and digit 5 but again the most consistent finding was that of substantial overlap of representations (typically up to 5mm). The differences between this latter study and the pilot study presented here may be accounted for by the number of subjects studied (26 subjects) and the slightly higher spatial resolution of the Beisteiner study (2mm X 2mm X 3mm). What is clear from these studies and others (Grafton 1993, Rao 1995, Sanes 1995, Dechent 2003, Sanes 2000, Schieber 2001, Sanes 2001) is that the motor cortex does not display the simple somatotopic organisation found in the sensory cortex. In addition there is good evidence for a gradual overlapping somatotopic arrangement in the motor cortex with a gradient moving from the legs at the vertex to the hands at the more rostral parts of the motor strip.

This greater degree of complexity within the motor cortex would appear reasonable if we consider in more detail the functions of the motor cortex. On the most basic level the motor cortex provides movement yet its function seems to be greater than just being a bank of upper motor neurons. Experimentation by a variety of investigators have shown that neuronal populations in the motor cortex encode for force (Evarts 1968), direction (Georgopoulos 1982, Georgopoulos 1988), speed (Moran 1999) and also goal direction (Alexander 1990) in reaching movements. In addition more recent studies in primates have suggested that the motor cortex and pre-motor cortex together form a single map used in the generation of complex movements (Graziano

2002, Krauzlis 2002). The motor cortex is also the site for a convergence of movement functionality that facilitates control of many different muscles in producing a single movement. As such, a rigidly separated somatotopy within the motor cortex would be unable to sub-serve these functions and, therefore, a more distributed organisation is likely and is observed (Sanes 2001).

Presumably, as techniques become more refined and experimental design more complex, the details of the functional organisation of the motor cortex will become transparent.

Given the discussion above, it is not surprising that the first pilot study investigating possible somatotopic organisation of flexion and extension movements failed to demonstrate any differences in the motor representations. This suggests that the organisation of within digit movements is at least as complex and distributed as that of individual digital representations. It is, however, the motor cortical control of agonists and antagonists that will have to be studied if dystonia is to give up its pathophysiological secrets.

The two other findings of these pilot studies come from the analysis of the motor activity in the dystonic subjects as compared to controls. It is clear that the number of patients studied is insufficient for statistical comparison. However, the observation that peak motor activity is reduced and the tendency to excessive spatial activity in the motor cortex are in keeping with and confirms the views presented in chapter 4.

6.5. Conclusions

In control subjects there is no discernible difference in the magnitude or location of motor cortical activity associated with flexion and extension movements of a single digit. In addition, individual digit 1 and digit 5 motor representations are overlapping and can not be discriminated from one another in both control and dystonic patients.

There are no significant differences in the positions of the motor representations of the individual digits in dystonic subjects when compared to controls.

There are quantitative differences between the two groups, the dystonia group showing a trend for reduced peak activation and increased spread of activation (size of representation) in the motor cortex. However, this did not reach statistical significance between the two groups.

Chapter Seven

Thesis Summary & Future Developments

7.1. Thesis Overview

The experiments presented in the preceding chapters have sought to investigate cortical activation abnormalities in dystonia. Focal dystonia has been utilised as a model of primary dystonia and a number of abnormalities in cortical activation have been shown during the preparation for movements as well as during their execution. Abnormalities within the spatial organisation of both sensory and motor cortices have also been demonstrated.

A brief global review of the abnormalities of cortical activity is summarised below, along with a discussion of possible pathophysiological mechanisms that might underlie focal dystonia. Finally, thoughts regarding the limitations of the presented studies and potential new lines of research are discussed.

7.1.1. Motor Preparation Abnormalities

Results from the experiments presented in chapters three and four confirm that in dystonia there is a general over activation of structures associated with the planning of movements, namely the Pre-SMA and the lateral premotor cortex.

Taking the results from chapter three and four together, there is a gradient of preparation abnormalities: preparation for button pressing (the no-go task) produces no abnormal activation of the Pre-SMA or LPMC. However, on preparing to draw a line the Pre-SMA becomes significantly over-active and in preparing to write, the Pre-SMA and the LPMC are both over-active.

These results suggest a thresholding effect. As the patient prepares for increasingly 'difficult' tasks there is a paralleled overactivity of the frontal striatal projections in individuals with focal dystonia. What factors govern this effect is unclear from these experiments, but the 'probability' of dystonia producing task maybe one factor. Of course, the experiment in chapters 3 & 4 do not address the issue of more complex tasks not involving holding a pen/pencil.

If we consider that the over-activation of an area is caused by excess neuronal firing (metabolic demand) in that area, this firing can be excitatory or inhibitory. Thus, overactivity could therefore be a primary phenomenon caused by abnormal 'wiring' or integration of these areas causing the generated plan to be complex and hence generating dystonia. Conversely, the overactivity could be a secondary problem caused by the complex planning required to perform a task with a dystonic hand. It seems unlikely that these areas are intrinsically abnormal in dystonia as they aren't macro or microscopically changed, and it is clear from chapter three that the activity is attenuated by the ongoing task. It therefore appears more plausible that the frontal striatal projections from the basal ganglia become more overactive when a complex (or specific) movement is planned.

The other abnormal activity seen during preparation in (i.e. the motor cortex) is discussed in section 7.1.2.1.

7.1.2. Output, Input and Integrative Abnormalities

7.1.2.1. Motor (output) abnormalities

Chapter four demonstrates that there are abnormalities in the motor cortex even during preparation. It appears that, normally, prior to a task, the motor cortex becomes active. This maybe due to motor imagery or ‘preparation’ of the cortex for the forthcoming action as patients had prior warning of the action in this paradigm. From the results, this ‘priming’ of MC is abnormal in dystonia and it continues to be abnormally underactive throughout actions.

Results presented in chapters three, four and six during activity reveal consistently that the motor cortex, has an abnormal distribution of activity across the wide areas of the motor cortex and the central peak of this activity has been reduced. Chapter six didn’t show an abnormal organisation of digit representation in the motor cortex of dystonic subjects compared to that of the controls.

Again, it must be remembered that the activation seen in the motor cortex is a reflection of neuronal activity, both inhibitory and excitatory. The relative composition of the extended area of activity is not known. This finding, however, could be interpreted as the fMRI equivalent of ‘overflow’ that is demonstrated with neurophysiological methods (Cohen 1988, Ridding 1995). The question again as to whether this is a primary (causative) or a secondary (to basal ganglia outflow) abnormality in focal dystonia is of considerable importance.

As mentioned in chapter 4, when considering the activation of the motor cortex by the basal ganglia, it is helpful to consider the ‘centre surround theory’ (Mink 1996). When a voluntary movement is made, the cortical areas send a signal of movement to the STN. The STN becomes active and, through its connections, causes excitation of the GPi. As the output of the GPi is inhibitory, there results, an inhibition of the

thalamus. In parallel to this pathway, a slower and more complex signal is sent to the striatum from the cortex. This message is processed by the 'integrative neuronal mechanisms' of the striatum and the resultant focal output is signalled to the GPi. This inhibitory input to the GPi focally disinhibits the desired motor programs whilst the undesired programs remain inhibited by the excitatory input from the STN. (See figure 7.1)

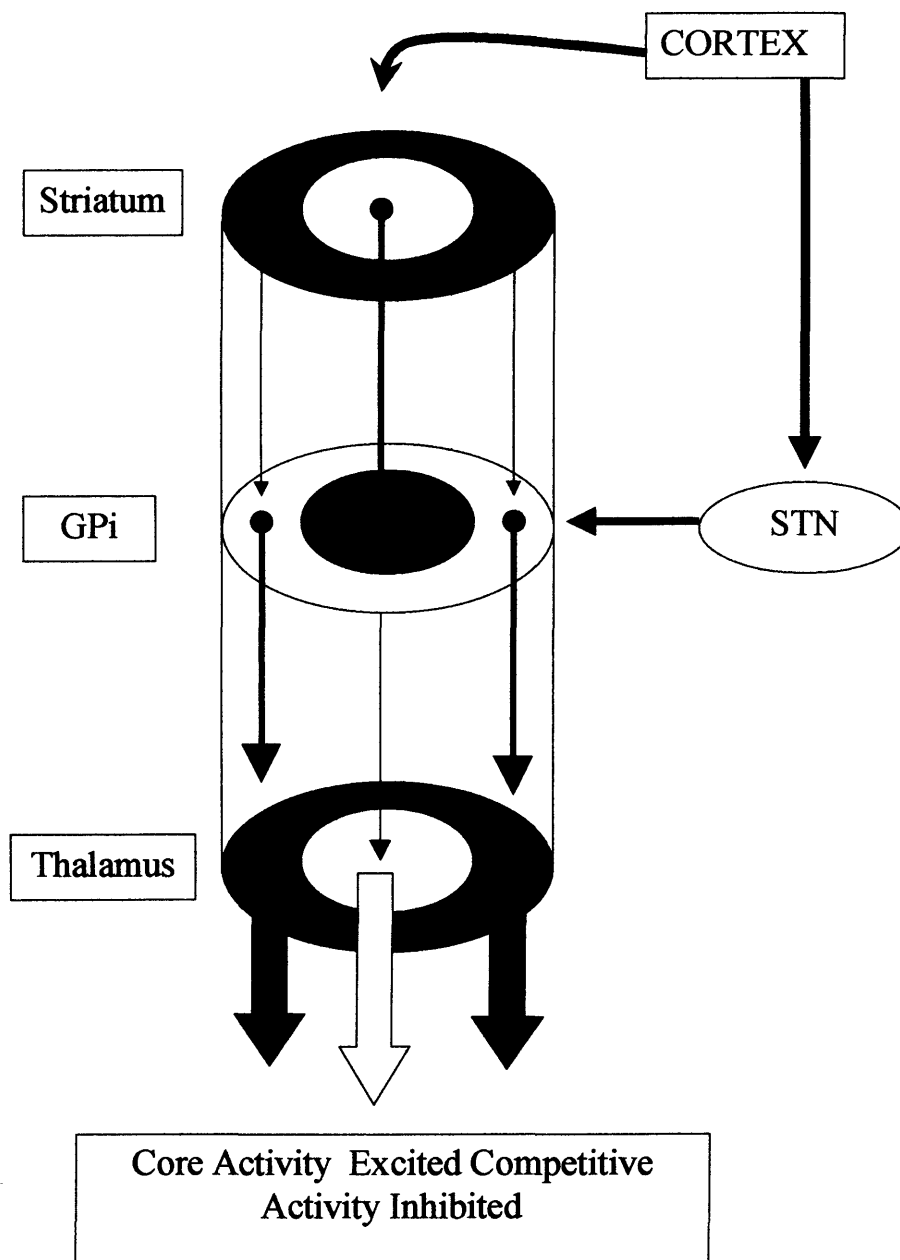


Figure 7.1. Centre-surround theory in normal individuals.

In dystonia, a fundamental malfunction of this theory can be proposed. If there were to be a blurring of the boundaries of the central 'core' desired activity and the surrounding undesired actions the resultant motor program will be run with competing undesired movements, such as the agonist/antagonist movements seen in dystonia. (See Figure 7.2).

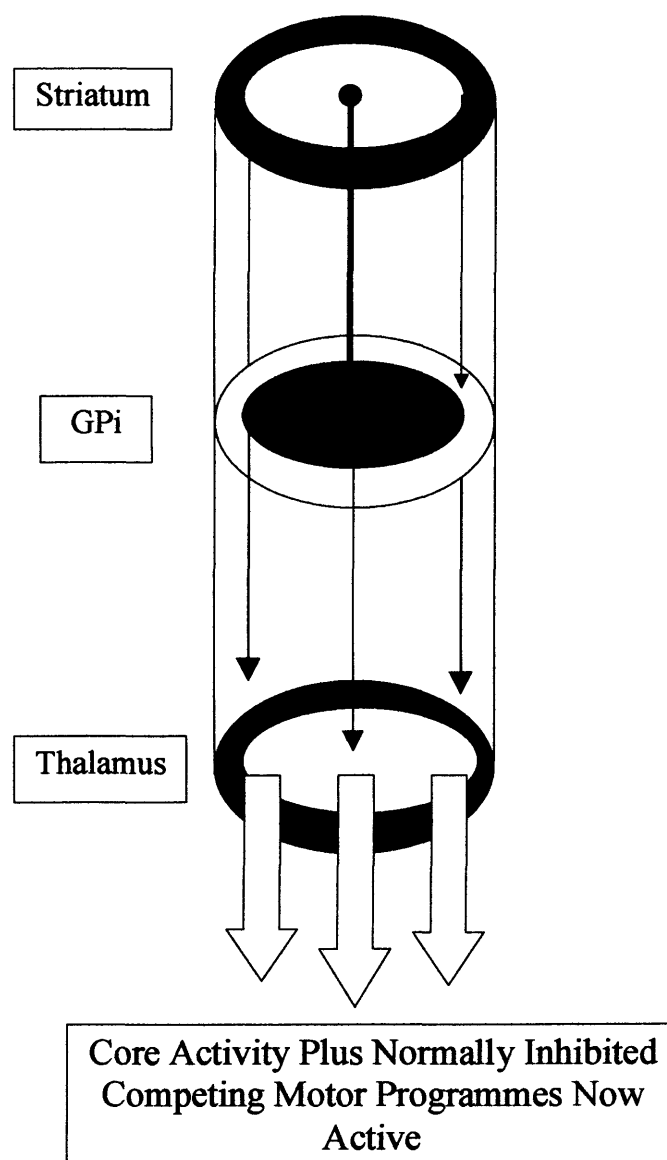


Figure 7.2. The centre-surround theory in dystonic individuals.

This type of theory alone cannot explain the motor cortical abnormalities found in these studies. If, however, the normal ‘focusing’ function of the striatum is impaired, the striatal output may actually ‘balloon’ out to encompass a wider area of activity hence activating wider regions of the motor cortex as depicted on Figure 7.3.

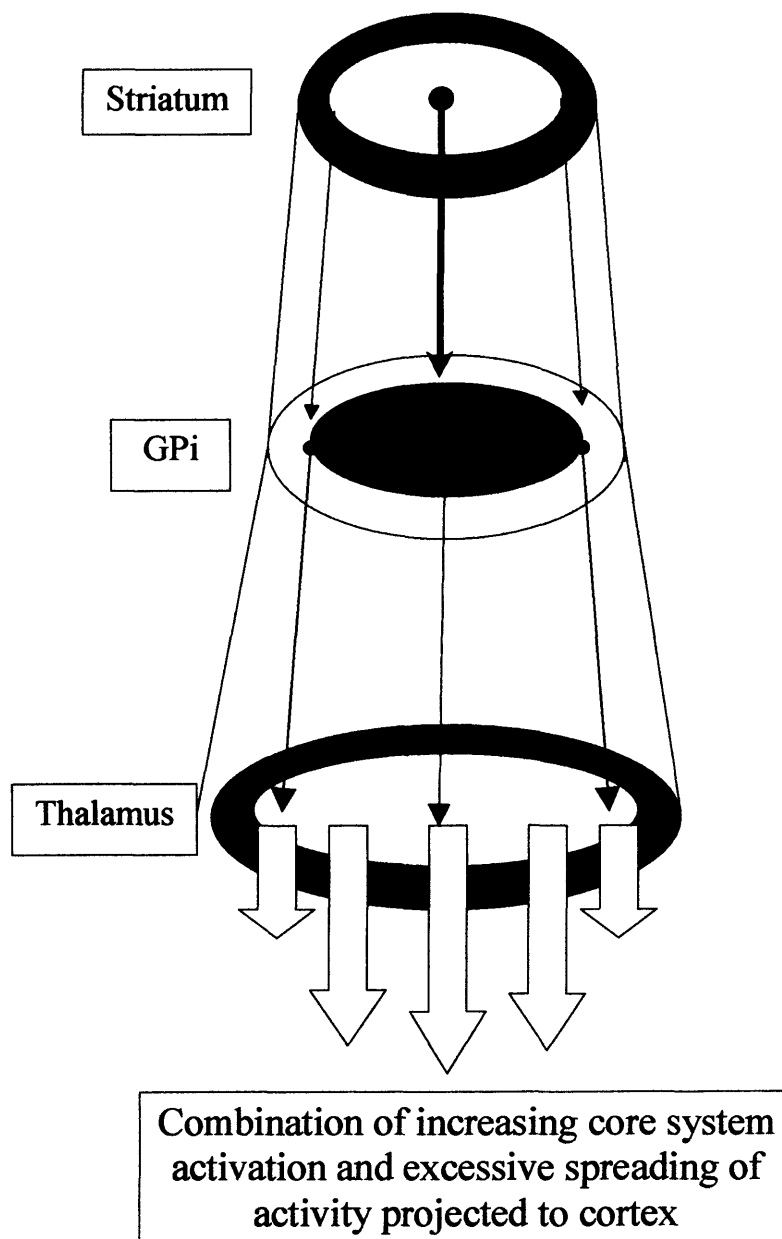


Figure 7.3. Showing excessive spatial overflow of activity from the striatum.

Another issue that isn't addressed by this model is the abnormalities in the temporal dynamics of this circuit. What would be the effect of the excitatory and inhibitory influences circulating through this system at differing rates? Presumably, if this temporal disturbance were in the order of milliseconds, it would cause a chaotic disruption to the timing and therefore sequencing of muscle activation as seen in dystonia. This, in theory at least, could lead to co-contraction of agonist and antagonist muscles that characterises dystonia.

Although this theory is helpful in the construction of how dystonic movements might occur, it does not help with the possible site of pathology in dystonia. It is possible that abnormalities from one or many of all the 'levels' (cortical, striatal, STN or thalamic) maybe contributory. Clearly, dystonia could be a 'final common pathway' result of lesions in any one of these areas. Given the multi-modal evidence from structural, functional and chemical studies, it would appear that the striatum is the most likely seat of the abnormalities and the widespread cortical changes a reflection of its dysfunction. A more precise understanding of the functional relationships of the basal ganglia and cortical areas will provide vital information to further our comprehension of dystonia pathogenesis.

7.1.2.2. Sensory (input) abnormalities

Chapter five illustrates that the discrete organisation of the sensory cortex is disrupted in focal dystonia. Chapters three and four illustrate no significant difference in the activity of the sensory cortex during the execution of the button-pressing task, the remaining still task and line drawing task. However, during the writing task the sensory cortex becomes over active.

It is of interest to hypothesise that one possible mechanism of dystonic contractions relates not just from the formation of the motor program but also to its ongoing

management. Sensory information collected from an ongoing movement is important in monitoring the movement, so that it can be compared and contrasted to the intended movement. If this sensory information is presented in an abnormal fashion, the consequent motor adjustments (even within a normally functioning motor system) will be misguided and potentially competitive to the ongoing movement. This hypothesis is interesting in that it may explain why a movement in dystonia can be initiated normally but later, as the movement progresses, dystonia develops. For example, it may take a number of lines before some patients with writer's cramp develop the cramping.

The existing literature also suggests that the abnormalities in the sensory system are part of the primary disorder, rather than a consequence of the dystonia. Studies showing bilateral sensory dysfunction in patients with unilateral dystonia (Tempel 1993, Meunier 2001) or even in asymptomatic carriers of DYT genes (Eidelberg 1998) suggests that there may be a sensory 'trait' (Tinazzi 2003) that requires further triggering from peripheral mechanisms such as trauma (Jankovic 2001) or overuse (Byl 1996) to become manifest.

7.1.2.3. Sensorimotor Integration Abnormalities

It is clear from the work present in this thesis and the vast array of other work (see section 1.6) that there are abnormalities of sensory input resulting in excessive and distorted sensory information being produced. In addition, there is also a wide variety of motor output abnormalities resulting in an uninhibited, poorly contained and chaotically sequenced motor plan. Which (if any) of these abnormalities is the prime mover in the dystonia pathogenesis is uncertain. Perhaps the next step in trying to clarify this situation is to understand how the sensory and motor information is integrated. The exact site of sensori-motor integration is not certain, and the two

systems converge at numerous levels in the nervous system, including the spinal cord, brainstem, basal ganglia, thalamus and cortex. Because of the widespread nature of the abnormalities, it seems likely the basal ganglia (Tinazzi 2003) with its vast connections would be the most likely area of abnormality. The sensory role of the basal ganglia has not been studied widely, but it appears that the basal ganglia selectively filters sensory information that seems relevant to movement control (Kaji 2001) and also filters expected sensory information as a consequence of movement in preference for novel sensory inputs (Lidsky 1985). Lidsky proposed that the basal ganglia may act to convert sensory information from a receptor oriented form to a form more relevant for guiding movement. More work to define the integrative role of the basal ganglia may help us define its pathogenic role in dystonia.

In conclusion, at the moment it is best to think of dystonia as not just as a motor disorder but also a somatosensory disorder.

7.2. Limitations of Thesis

It is important to note that there are a number of limitations with the studies presented in this thesis.

7.2.1. Patients studied

In an ideal situation it would have been preferable to study patients with a whole range of dystonic conditions. For the reasons outlined in section 2.8.2, this was thought not to be technically feasible. This of course implies that the results of these studies can only be applied to the patients with focal hand dystonias rather than focal dystonias generally.

7.2.2. Areas Scanned

The areas that have been scanned have been limited in all the studies. In particular, the prefrontal areas, the basal ganglia and the cerebellum have been somewhat overlooked. Unfortunately, due to the distortions and loss of resolution that accompany rapid scanning, detailed study of these areas in all subjects was not possible. Self imposed time limitations on the length of experiments also meant that compromises on the extent of imaging had to be made; however, this approach is thought to be justified in terms of the accuracy and compliance achieved with the paradigms. It is the case that longer paradigms produce a greater number of mistakes and hence contamination of results.

7.2.3. Analysis

It would have been preferable to analyse some of the studies above with SPM statistical packages especially to compare the results with the types of analysis used. The development of SPM analysis for MRI studies has occurred in parallel with these studies and during the analysis, SPM was not as developed or even as available for fMRI analysis as it is today. In a few cases SPM 97 analysis was performed on the data, and similar but more significant result were obtained. However, more detailed spatial analysis was not able to be performed using this program.

7.2.4. BOLD response

The bold response is known to be a reflection of blood flow, volume and oxygenation. This response is thought to reflect neuronal activity of a particular area. However, whether the bold response is linear in response to increasing activity and how the bold response performs at very high levels of activity are less clear. Therefore, the results

have been interpreted carefully and constrained against other functional imaging modalities in order to protect against over or understating the findings.

7.2.5. Temporal resolution

The time that exists between repeatedly scanning the volume of brain (the TR) provides an ‘un-sampled period’ in all fMRI studies. It is possible that during this period there are changes in the BOLD effect that cannot be detected (i.e. higher peaks and troughs or rapid activations). Interpretation of the studies should have this intrinsic failing of fMRI borne in mind.

7.2.6. Contamination of activations

Although attempts were made to separate out preparatory and execution activity, contamination of these states could occur especially in the patient groups when dystonia was induced. It is not known how long the cortical changes associated with dystonia will take to resolve and there is some emerging evidence that this may be longer than previously thought (Blood 2004).

7.3. Present & Future Developments

MRI techniques continue to develop at an astounding pace. During the course of this project, a number of new techniques have been described and become more widely available. In addition, more refined image processing and statistical analysis is now possible. With more experience in paradigm design, our understanding of normal brain function continues to improve and theories of movement generation become more integrative and complex. These continuing developments mean that a greater understanding of movement and its dysfunction are now or will soon be possible. In respect to pathophysiology of dystonia, a number of aspects are amenable to further study.

7.3.1. Role of the Cerebellum

There is some evidence for the dysfunction of the cerebellum in dystonia. Cerebellar abnormalities have been known to cause focal dystonia (LeDoux 2003, Alacron 2001, Cockerell 1996, Tan 2001, Alafaci 2000). In addition, studies on primary dystonia have often implicated the cerebellum (Ceballos-Baumann 1997, Playford 1998, Eidelberg 1995, Eidelberg 1998, Hutchinson 2000). The studies presented in this thesis didn't image the cerebellum in any detail because of the distortions produced in this area during rapid EPI sequences. Using surface coils and other sequences, this area could be functionally imaged and may prove an interesting area of further study as the cerebellar connections to the basal ganglia and thalamus may well be important in the pathophysiology.

7.3.2. High Resolution Structural Imaging

The resolution of MRI techniques continue to improve, techniques with resolution of a third to a quarter of a millimetre are currently in development. Structurally, further imaging of the striatum may prove fruitful, as this site is the only area of the brain in which potential super-structural abnormalities have been found. High resolution imaging of the motor structures would also be worth considering, ascertaining if any intrinsic abnormalities of structure or neuronal organisation could be found in these areas.

7.3.3. High Resolution Functional Imaging

More detailed functional imaging, especially of the motor structures, will further our understanding of not only how movements are represented in the motor cortex in normal subjects, but how the basal ganglia triggers them. Already, some progress has been made in the functional MRI of the basal ganglia (Blood 2004) and high-

resolution studies of the motor cortex are being planned. These and other studies may well further our understanding of the many roles these structures perform and allow us to theorise as how these structures abnormally function in dystonia.

7.3.4. The BOLD Effect and Non-Contrast perfusion Imaging

Even with the widespread use of fMRI in research and indeed within the clinical setting, the accusation of ‘running before we can walk’ can be levelled at fMRI. Our understanding of the BOLD effect is still not complete, and therefore interpretation of results must be tempered with caution. As part of this thesis, work was undertaken in collaboration with others (Pears 2003, Francis 2003) to further our understanding of the mechanisms contributing to the BOLD effect. This work will not be presented here, but essentially explores the contributions of cerebral blood volume and venous oxygenation to the cerebral activation.

Because of the potential limitations of the BOLD effect, newer MRI techniques such as arterial spin labelling can be employed. This technique is a powerful non-invasive method for measurement of CBF that does not require the injection of contrast agent. Rather, this approach uses blood water as an endogenous contrast agent for the measurement of perfusion. During the course of this project, preliminary experimentation was begun using the technique of EPSTAR (Edelman 1994), to study both normal and dystonic patients, during a task of increasing severity structured to ensure dystonic cramping in patients. Analysis of this data has proven very difficult as no standard statistical method for comparison of this kind of data and this type of time series has been developed. Attempts to develop a system of analysis were advanced, but this couldn’t be completed due to time constraints. Further work and analysis will continue, in order to plot dynamic changes of blood flow in cortical areas that will directly reflect the genesis of a severe dystonic cramp.

7.3.5. Tensor imaging

This adaptation of MRI has been developed to study the tissue structure and orientation of brain tissue. It allows information to be gathered about the brains connectivity rather than the function so-called: ‘MR tractography’(Bammer 2003). It is clear that if we are to imply function of brain areas, knowledge the connectivity of these areas will be vital to the construction of and overall hypothesis of dysfunction in dystonia.

7.3.6. Multimodal Approach

From the evidence and experiments presented in this thesis, it is clear that no single approach to the investigation of dystonia will unveil its pathophysiology. fMRI alone is not yet a powerful enough technique to study all the aspects that are required to reveal a full picture of the pathogenesis of dystonia. Multimodal approaches will have to be developed combining methods of investigation from the genetic molecular level to complex hybrid imaging procedures.

7.3.7. Patients

There is now growing evidence that, although phenotypically primary dystonias look very similar, they may have different pathological substrates. It is best therefore to plan future experiments to be conducted with very similar and perhaps genetically identical groups of patients.

By performing longitudinal studies involving the identification of presymptomatic individuals and repeatedly studying them as they become symptomatic, more useful information could be gained. These studies should include analysis of the potential factors (environmental or otherwise) that define the change from the asymptomatic

state to a symptomatic one. At present there is no way of defining a group of patients, as even the patients with a genetic predisposition to dystonia (e.g. DYT-1) have no guarantee of becoming symptomatic.

These and similar obstacles will have to be met and overcome if the mysteries of dystonia are to finally be unravelled.

Appendix 1

Samples of the forms used for volunteer information volunteer consent and forms used to record scanning parameters.

Nottingham University Magnetic Resonance Centre

Volunteer Safety Form

Volunteer number: Number of scan:.....

Name:

Address:

Phone number: Date of birth:

Have you read the Dystonia study information sheet? YES NO

Have you had opportunity to ask questions and discuss the study? YES NO

DO YOU HAVE ANY METAL IMPLANTS? YES NO

Do you have a pacemaker or artificial heart valve? YES NO

Do you have aneurysm clips in your head? YES NO

Have you ever had any metal fragments in your eyes/body (e.g. shrapnel)? YES NO

Have you had any recent surgery? YES NO

Have you ever had hearing problems or ever worn a hearing aid? YES NO

Do you have any dentures, dental pins or metal brace? YES NO

Do you have any artificial limbs? YES NO

Do you have any known medical complaints/conditions? YES NO

Are you taking any medication? YES NO

Are you claustrophobic? YES NO

Is there a possibility you may be pregnant? YES NO

Do you agree to take part in the study? YES NO

Please note you are free to withdraw from the study at any time, without having to give a reason and that answers and results are held in strictest confidence.

Signed

Date:

NAME (BLOCKCAPITALS):

UNIVERSITY OF NOTTINGHAM – MAGNETIC RESONANCE CENTRE

CONSENT FORM

Study: Functional Magnetic Resonance Imaging in Dystonia.

Investigators: S. Butterworth, A. Peters, P. Gowland, P.G. Morris,
G.V.Sawle.

Checklist:

Information sheet	☐
Safety form	☐
Questions & Further Information	☐
Withdrawal at any time	☐

Consent:

I, (print name) hereby consent to take part in
the above study, the nature of which has been explained to me verbally in writing and
verbally by

I understand I am free to withdraw from the study at any time, without giving a
reason.

Signature.....

Investigator

Date of scan:

You should not have participated in any other research project in the last three
months.

FUNCTIONAL MAGNETIC RESONANCE IMAGING IN DYSTONIA INFORMATION SHEET

What is functional Magnetic Imaging?

Functional Magnetic Imaging is a technique for investigating how the brain works via the natural changes in blood oxygen levels and local blood flow and volume, which occur during neural activity. Many studies using this technique have been performed both at the Magnetic Resonance Centre and in other centres throughout the world. Typically fast scanning of the brain is undertaken while subjects perform a simple task such as button pressing or writing. Using a statistical analysis of the pictures acquired during scanning, areas of the brain specifically used during the task can be visualised. We hope to use this technique to determine which areas of the brain are responsible for producing Writer's Cramp and other Dystonias.

What will it involve for you?

You will be asked to attend the Magnetic Resonance Centre for approximately seventy-five minutes during which time a quick assessment of writing will be performed. This will be followed by the scan of your head. You will be asked to lie in the scanner for a period of no longer than 45 mins. Whilst you perform a simple task such as writing your name, the scanner will take a lot of pictures of your head. If at any time you wish to withdraw from the study you are free to do so. You can even stop during the scan if you wish.

Are there any risks?

Unlike other methods of scanning at the brain functional MRI does not involve any injection or X-rays as it is based around magnetism and radio waves that are considered to be biologically safe. However, because a very strong magnetic field is used there are potential problems if there is any metal in your body such as shrapnel, aneurysm clips or metal implants; the magnetic field could affect them and make them move, which could cause pain and is potentially dangerous. If you have any metal in your body it is best to avoid being scanned.

Occasionally some individuals feel dizzy when they first lie in the scanner.

The MRI scanner is noisy and because the space inside the scanner is quite small some people feel claustrophobic, though these can be overcome somewhat by using earplugs and by making it possible for you to see out of the end of the scanner.

The Data Protection Act requires that volunteers should be made aware that data concerning them is being held on computer.

Appendix 2

List of publications from research

Papers

- 1) Butterworth S, Francis S, Kelly E, McGlone F, Bowtell R, Sawle GV. Abnormal cortical sensory activation in dystonia: an fMRI study. *Mov Disord.* 2003;18(6):673-82.
- 2) Pears JA, Francis ST, Butterworth SE, Bowtell RW, Gowland PA. Investigating the BOLD effect during infusion of Gd-DTPA using rapid T2* mapping. *Magn Reson Med.* 2003;49(1):61-70.
- 3) Francis ST, Pears JA, Butterworth S, Bowtell RW, Gowland PA. Measuring the change in CBV upon cortical activation with high temporal resolution using look-locker EPI and Gd-DTPA. *Magn Reson Med.* 2003;50(3):483-92.
- 4) Newton J, Sunderland A, Butterworth SE, Peters AM, Peck KK, Gowland PA. A pilot study of event-related functional magnetic resonance imaging of monitored wrist movements in patients with partial recovery. *Stroke.* 2002;33(12):2881-7.
- 5) Peck KK, Sunderland A, Peters AM, Butterworth S, Clark P, Gowland PA. Cerebral activation during a simple force production task: changes in the time course of the haemodynamic response. *Neuroreport.* 2001;12(13):2813-6.

Abstracts

Nine abstracts on all aspects of this work were presented at international meetings.

Presentations

I have presented this work during platform presentation at the Human Brain Mapping conference in Düsseldorf and at the Philadelphia meeting of the International Society of Magnetic Resonance in Medicine.

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